Process Analytical Technology in Biopharmaceutical Manufacturing

by

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Submitted to the Department of Chemical Engineering and the MIT Sloan School of Management in partial fulfillment of the requirements for the degrees of

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Abstract

Process Analytical Technology (PAT) became a well-defined concept within the pharmaceutical industry as a result of a major initiative by the FDA called "Pharmaceutical cGMPs for the 21st Century: A Risk-Based Approach." The FDA defines PAT as "a system for designing, analyzing, and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality." The biotechnology industry has started incorporating PAT in manufacturing, because of regulatory pressure and because the previous blockbuster-oriented business model is becoming less viable.

This thesis proposes a methodology for evaluating PAT systems and delivers guidance on how to develop and implement them to effectively manage risk in biopharmaceutical manufacturing. The methodology includes guidance regarding identifying opportunities, evaluating and implementing novel analytical technology, appropriately applying acquired data, and managing change associated with PAT implementation.

Experimental results from a novel PAT system that acquires light scattering and UV absorbance data to control chromatography during large-scale manufacturing are presented as a case study. The case study follows the methodology to show how a system optimized for a laboratory can be scaled for use in biopharmaceutical manufacturing.

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Chapter 1

Introduction

1.1 Project Motivation

Historically, the biopharmaceutical industry has focused its operational efforts on capacity and quality, but that mindset has been changing over the last decade. Revenue pressures ranging from patent expirations to global competition have driven maturing biopharmaceutical companies to seek innovative ways to continue delivering value to shareholders and society. These changes in the industry's landscape have caused these companies to turn to a relatively untapped source of competitive advantage: operational excellence, which includes improved productivity, risk management, and safety. In more mature industries, companies have significant experience extracting the benefits that operational excellence provides, but biomanufacturing is still in the early stages of identifying and taking advantage of this source. Process Analytical Technology (PAT) is one component of the operational excellence toolset that promises to reduce biomanufacturing costs, enhance quality, and increase process knowledge.

Regulatory agencies have also played a key role in fostering the adoption of Process Analytical Technology by both the biopharmaceutical and traditional pharmaceutical industries. In direct response to the hesitancy of pharmaceutical companies to adopt innovative operational paradigms, the FDA chartered an initiative, "Pharmaceutical cGMPs for the 21st century - A risk based approach," through which it formally communicated guidance to encourage the future use of PAT in bioprocessing. The

FDA is not alone, however, as other agencies such as the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and the European Medicines Agency (EMA), among others, have independently issued guidance regarding PAT.

Many pharmaceutical companies have chartered efforts to incorporate principles of Process Analytical Technology into their operating organizations, but at the time of this writing these efforts have not yet achieved the results and widespread adoption originally envisioned. Several factors have influenced this, including the difficulty of making significant changes to existing pharmaceutical operating facilities, the challenges of changing industry and corporate culture, and the need to develop organizational capabilities to effectively integrate PAT in biopharmaceutical operations.

1.2 Problem Statement

The current interest in Process Analytical Technology has spurred a spate of new tools and systems from both entrepreneurial ventures and established companies. In addition, biomanufacturers are evaluating the potential of adapting methods formerly confined to research applications to use as PAT in bioprocessing. In spite of the advent of these novel technologies, biomanufacturers have encountered the problem of understanding precisely what problems PAT needs to solve and which applications will yield benefits in their processes with their products. Consequently, many organizations have chartered working groups and departments to clarify how to best address perceived shortcomings.

This research intends to aid in resolving this problem by providing a methodology for biomanufacturers that can assist in identifying, evaluating, and implementing currently available PAT. We also intend to highlight opportunities where a PAT solution could be engineered from current technology, and areas where PAT realization lies a few years in the future. In an effort to lend credibility to this research, a case study of the development of a particular PAT system is included to demonstrate how a laboratory concept can be converted to an implementable biopharmaceutical

manufacturing solution.

1.3 Thesis Statement

The thesis proposed in this work is two-fold. First, it asserts that a structured methodology, detailed herein, for incorporating PAT in biopharmaceutical manufacturing will yield the benefits of reduced costs, enhanced quality, and increased knowledge associated with effective operational excellence. This structured approach focuses on identifying commercially available systems to satisfy the defined needs, assessing where gaps exists, making plans to fill the gaps, and then prioritizing development efforts.

Second, the thesis defends the aforementioned approach by proposing an example of PAT, which includes a novel approach for analyzing and controlling chromatography to a desired level of aggregated protein species in a biomanufacturing process. This example will also demonstrate preliminary evidence of the system's benefits, while including discussion of areas of further research and development.

1.4 Research Methodology

This research was conducted primarily at Amgen, Inc., in Thousand Oaks, CA, and the large-scale experiments for the PAT system were carried out in a pilot plant facility on Amgen's campus. The entire engagement with Amgen from project concept to completion was 6 months. Because of the relatively compressed time frame, this research is not intended to be a comprehensive body of research comprising the entirety of PAT in biomanufacturing. Rather, it is intended to use a representative case study in coordination with a review of the literature regarding PAT in biomanufacturing to support the thesis presented. The approach taken to the research was a cycle of the Plan-Do-Check-Act (PDCA) continuous improvement approach popularized by Deming. We consider this approach appropriate because undertaking the research with this methodology encourages the cycle of continuous improvement to carry on

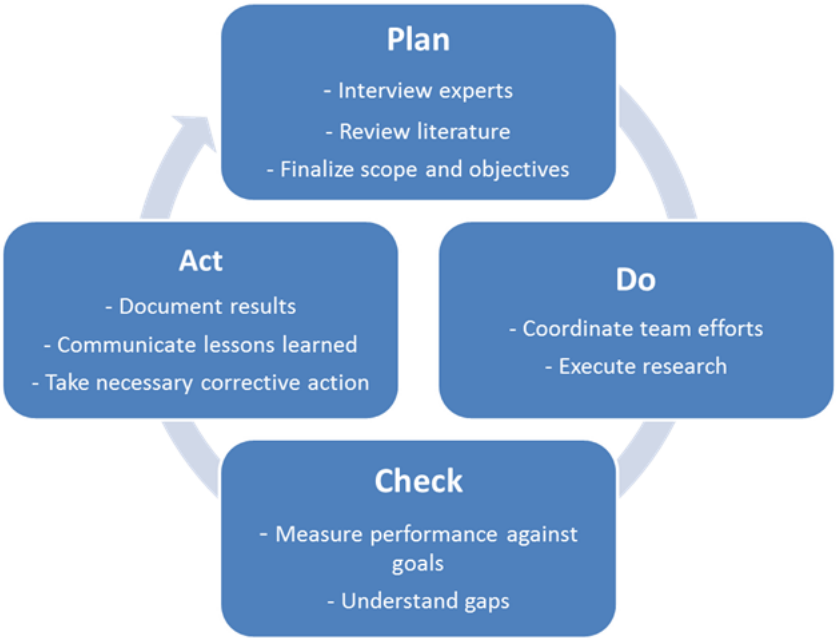

Figure 1-1: Plan-Do-Check-Act framework for our research

beyond this particular thesis. A brief description of the major activities undertaken in the Plan-Do-Check-Act framework is shown in Figure 1-1, and specific steps regarding the PDCA approach for the case study portion of this work will be further defined.

During the Plan phase, the novel framework for incorporating PAT in biomanufacturing was developed based on interviews and meetings with over 20 scholars and professionals involved with PAT from various areas of expertise. These scholars and professionals include experts knowledgeable in management, traditional—or "small molecule"—pharmaceutical operations, biopharmaceutical operations, process control and automation, optical science, protein aggregation, chromatography, biological analysis, chemical analysis, and others. Previously published literature was reviewed to understand the relevant research, with an emphasis on the areas of Process Analytical Technology, biomanufacturing, protein aggregation, online process analysis, and biopharmaceuticals. Additionally, the scope and objectives of the research were aligned with the project objectives of Amgen, the sponsoring entity, prior to executing research and development. In all cases, data and information are presented only in sufficient detail to substantiate and illustrate the results without compromising

information deemed proprietary by the sponsor.

In the Do phase, a team was assembled with representation from functional expertise areas including light scattering detection, process control, protein purification, equipment engineering, wiring, and input/output (I/O) for the novel PAT system. The PAT framework also integrated input from the aforementioned team and included input from project management leaders, quality leaders, and manufacturing leaders responsible for incorporating PAT into their operating organizations. The efforts of the team were coordinated to best meet the needs of the project. The research was executed as described in the PAT framework and case study.

For the Check phase, performance was measured by whether the novel PAT system could satisfactorily measure and control the amount of aggregated protein species in the chromatography pool. In addition, the PAT framework was measured on its merit as a simple communication tool and plan for deployment in a biomanufacturing organization intending to incorporate PAT principles in operations.

In the Act phase, results were communicated to the sponsor company and presented for this thesis. These communications are intended to provide insight into future opportunities for PAT in biopharmaceutical manufacturing.

The primary test of this research methodology is the specific example regarding manufacturing chromatography column analysis and control described herein and the applicability of the PAT framework. The data for the chromatography experiments were collected from pilot-scale runs of a development-phase therapeutic protein—specifically a monoclonal antibody—in a facility dedicated to development and experimentation at scales larger than a typical laboratory can offer. These large-scale experiments serve as a basis for the claimed applicability of these results to other large-scale biopharmaceutical manufacturing processes.

1.5 Thesis Overview

This thesis is segregated by chapter, and the contents of each can be briefly described as follows:

Chapter 1 is an introduction, which includes the project motivation, problem statement, central thesis statement, and research methodology. We set forth the reasons for conducting this research and in the environment in which the research was carried out. Furthermore, we detail how the research was executed and what central hypothesis is to be tested.

Chapter 2 gives a background on topics relevant to the research including a literature review. The chapter includes descriptions of the biopharmaceutical industry, biomanufacturing, and Process Analytical Technology (PAT). These descriptions serve as a way to link the early history of biotechnology to biopharmaceutical manufacturing, and then to describe how biopharmaceutical manufacturing transitioned to an area of active operational improvement from a relatively inefficient—in terms of operations—early stage. In addition, the role of PAT in improving the state of biopharmaceutical manufacturing is explored.

Chapter 3 specifically describes the research findings related to incorporating PAT in biopharmaceutical manufacturing. It details a structured approach to developing and implementing PAT, and it includes a detailed example of an implementation in a large-scale experimental facility.

Chapter 4 outlines an investigation into possible future PAT development opportunities. It reviews current research in three primary areas: advanced optical technologies, microscale and nanoscale devices, and sources from other industries and disciplines. This chapter highlights key areas where PAT systems could be developed in the future.

Chapter 5 contains our recommendations for biopharmaceutical industry members who seek to incorporate principles of PAT in their operations. The emphasis is on identifying straightforward initiatives that industrial organizations can undertake to improve operational excellence.

Chapter 6 is a conclusion, which summarizes the body of work presented. It includes the implications of this research on the biopharmaceutical industry at large.

Chapter 2

Background and Literature Review

2.1 Biotechnology and the Biopharmaceutical Industry

The foundation of the biopharmaceutical industry is biotechnology, which is the use of naturally occurring or engineered living organisms to generate a desired output. Humans have employed principles of biotechnology, in the broadest sense of the term, to achieve a variety of ends for millennia, such as raising cows for their milk and cultivating crops. Therapeutic biotechnology involves employing the principles of biotechnology for improving an organism's quality of life, and a significant advance in the progress of therapeutic biotechnology occurred in the 1700s, when vaccines and vaccine production were developed. These vaccines eventually wiped out many widespread diseases. However, vaccination was limited to a set of diseases that could be isolated, inactivated, and injected in sufficient quantities to provide immunological defense to the targeted virus. In addition to the small set of immunizable disease using this technology, it was costly and inefficient to scale production. Because of these limitations, early vaccines are not generally included in the definition of biopharmaceuticals. The accumulated human experience and biotechnological knowledge from crops to vaccines ultimately did give rise to the modern biopharmaceutical industry.

Biopharmaceuticals, or biologic medical products, are therapeutic medicines pro-

duced by the cells of a living organism. Biopharmaceuticals differ from traditional pharmaceuticals in at least three key ways: production process, molecular structure, and method of patient delivery. The manufacturing process will be treated at length in a later section, but the most unique aspects of biopharmaceutical production are the steps required to culture organisms that produce the desired therapy. In essence, the production of the biopharmaceuticals is based on the ability to program an organism such as a cell, to rapidly and reliably replicate itself and then produce the desired molecule. In contrast, traditional "small molecule" manufacturing processes do not rely on a programmed organism as a means of production, but rather rely on reactions and separations of purified reagents to yield the desired product. The biopharmaceutical molecule is often significantly larger than that of a traditional pharmaceutical as shown in Figure 2-1*, and the resulting molecular complexity causes its shape and arrangement to impact its function within the human body as much as its molecular composition.

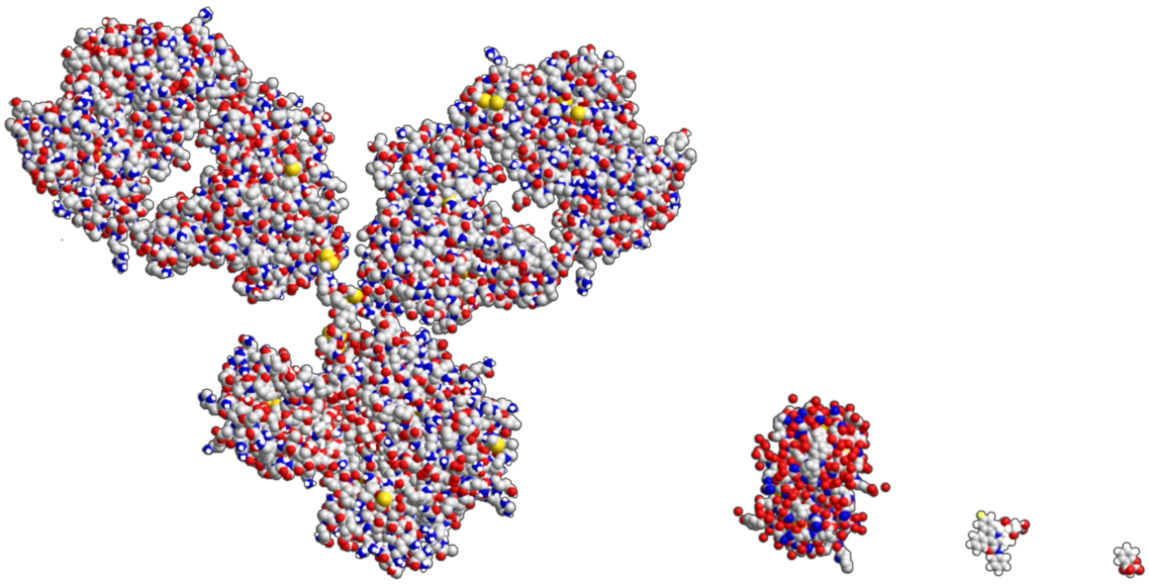

Figure 2-1: Illustration of therapeutic molecule sizes (not exact scale) which are, from left to right, a monoclonal antibody, epoetin alfa, loratadine, and acetylsalycylic acid (aspirin)

Because of this large, complex structure, biopharmaceuticals tend to be less stable

*The first two molecules in Figure 2-1, the monoclonal antibody and epoetin alfa, are considered biopharmaceuticals, while the latter two are considered traditional pharmaceuticals.

and less able to penetrate pores within the human body than traditional pharmaceuticals over a given period of time. Therefore, biopharmaceuticals are most often delivered parentally, or by injection, while traditional pharmaceuticals are often delivered orally. In spite of these differences, there are exceptions to these generalities, so they should be considered as guidance rather than strictly defined categories. Perhaps due to the difficulty in achieving economies of scale with existing development and manufacturing methods, until the middle of the twentieth century most synthetic therapies were developed and manufactured using more chemical, or "small molecule" methods.

Biopharmaceuticals as a class of therapies include all therapeutic products enabled by recombinant DNA and synthetic antibody technologies. Recombinant DNA technology, including the discoveries that led to it, was the major scientific breakthrough that facilitated the delivery and development of a variety of synthetic biopharmaceuticals at large scales. Recombinant DNA is defined as any synthetic DNA molecule, including molecules formed by separating and combining portions of DNA from one or various organisms. As discussed, prior to recombinant DNA technology, the only systematic and scaleable way of developing and manufacturing new human therapies was through the use of chemical processes or inactivating viruses. Therefore, desired pharmaceutical therapies, including proteins and antibodies, would either have to be synthesized chemically or extracted from organisms in vivo. The former continues to be cost prohibitive as it pertains to proteins and antibodies, and while the latter is of a more biological nature, its scale and scope are limited by the ability to discover existing proteins and antibodies of therapeutic significance through trial and error.

One of the first methods developed for the successful creation of recombinant DNA molecules was published in 1972,[1] and attempts to produce biopharmaceuticals using this new technology followed close behind. After several years of developments, these attempts culminated in a major success: the first biopharmaceutical to be developed, engineered, and manufactured using a biological—rather than chemical—paradigm for development and production. This first biopharmaceutical approved for human use was a synthetic human insulin analog, Humalog (insulin lispro), devel-

oped through the collaboration of Genentech Inc. and Eli Lilly and Company.[2] This technical and commercial breakthrough paved the way for significant investment in the biotech industry, which hit another milestone when Epogen® (epoetin alfa), by Amgen, became the first blockbuster† biopharmaceutical.[3]

The most common cells engineered to produce recombinant DNA protein therapeutics are derivatives of the mammalian cells of Chinese hamster ovaries (CHO) and the bacterial cells of E. coli.[4,5] For industrial processes that generate biopharmaceuticals on a large scale, the product is most commonly a protein, such as epoetin alfa. A significant subset of biopharmaceutical proteins is synthetic antibodies, including monoclonal antibodies such as adalimumab.

Monoclonal antibody technology for use in human therapeutics was the second major scientific breakthrough enabling the biopharmaceutical industry's growth. A monoclonal antibody is a synthetic, Y-shaped molecule derived from a single cell line that preferentially binds to a specific region of an antigen, which is a compound that is often a key factor associated with a disease. This binding is a critical part of the immune response for eliminating the threat of infectious bacteria and viruses. The development of monoclonal antibodies provided researchers with another broad platform to extend therapeutic research beyond replicating proteins that were biologically similar to those present in the human body, since monoclonal antibodies could be engineered to specifically target an antigen of interest. After many years of research and development, the first monoclonal antibody approved for human therapy was muromonab‡ in 1986.[6]

In summary, the combination of recombinant DNA and monoclonal antibody technologies provided the biopharmaceutical industry with greater ability to synthesize proteins with desired structures, properties, and therapeutic effects. Within this expanded realm of possibilities scientists have developed new and improved treatments for a variety of illnesses. Since the approval of synthetic human insulin, a series of successful biopharmaceuticals has generated a rapidly growing, profitable industry,

† "Blockbuster" indicates a therapy that has annualized revenue of over one billion dollars.

‡ Modern naming conventions stipulate that the scientific name of a monoclonal antibody end in "-mab", but these conventions were not in place when muromonab was developed.

Protein Name(s)	Trade Name(s)	Revenue ($B)	Company
Adalimumab	Humira	7.9	Abbott
Etanercept	Enbrel	7.4	Amgen, Pfizer
Infliximab	Remicade, Simponi	5.8	J&J, Merck & Co.
Bevacizumab	Avastin	5.7	Roche
Trastuzumab	Herceptin	5.7	Roche
Filgrastim, Pegfilgrastim	Neupogen, Neulasta	5.2	Amgen
Insulin glargine	Lantus	5.2	Sanofi-Aventis
Interferon beta-1a	Avonex, Rebif	4.9	Biogen Idec, Merck KGaA
Ranibizumab	Lucentis	3.7	Novartis, Roche

Table 2.1: Top-selling biopharmaceutical products of 2011 - data gathered from 2011 public financial reports

as shown by Table 2.1[§], which depicts the top-selling biopharmaceutical products of 2011.

2.2 Biosimilars

In addition to developing and offering different, competing treatments for similar ailments, the idea of developing and offering biologically similar treatments, or biosimilars moved from concept to possibility when patents on the earliest biopharmaceuticals expired. The advent of biosimilars could significantly impact the biopharmaceutical industry, because the business case for biosimilars is based on price competition. Therefore, developers and producers of biosimilars would have incentives to minimize facility and operating costs to the extent possible. Yet biosimilars have not been developed and approved in quantities comparable to generic versions of traditional pharmaceuticals. This lack of competition for products whose patents have expired has led to intensive communication between governments and the biopharmaceutical industry because societies tend to view this as a market failure.

[§]Different products may be developed to treat the same or similar conditions. For example, the top three products in Table 2.1, adalimumab, etanercept, and infliximab are all approved to treat rheumatoid arthritis and other autoimmune diseases.

At least one major reason behind the lack of competition is that the manufacturing equipment, host cell line, process design, and raw materials used for a particular biopharmaceutical are more important contributors to its therapeutic efficacy than those of a traditional pharmaceutical, which relies primarily on its molecular composition for efficacy.[7] Another major reason for the lack of commercial biosimilars was the absence of a regulatory pathway for the approval of these therapies. To address perceived regulatory shortcomings, the European Medicines Agency (EMA), issued a procedure for the approval of biosimilars in 2005.[8] Then, in 2012, the FDA issued its own guidance.[9] Since that time, a number of companies have announced their intent to capitalize on the commercial opportunity presented by biosimilars. These efforts generally have taken the form of an established biopharmaceutical company with a large, developed marketing and sales function partnering with experienced generic pharmaceutical manufacturers. For example, Pfizer and Biocon announced such a partnership in 2010, and Amgen and Watson followed suit in 2011.[10,11]

These partnerships lend credibility both to the claim that biosimilars are significantly less expensive to develop than original biopharmaceuticals, and that biosimilars increase pressure on biomanufacturing companies to explore opportunities to operate more efficiently. By some estimates, biosimilars cost $100M to $200M to develop, whereas novel biopharmaceuticals cost a significantly higher $1.2B.[12] Even though the future state of biosimilars is unclear at the time of this writing, the intent of stimulating increased competition could have a significant impact on biopharmaceutical manufacturing as companies look for cost effective ways to develop and manufacture biosimilars to compete with proven, profitable products.

While the biopharmaceutical industry continues to innovate, progress, and deliver therapies to patients, increasing competition including the threat of biosimilars is driving companies to continue seeking opportunities to deliver value to patients and shareholders. Maturing products and technologies have led to a need for more efficient operating processes, not only to meet capacity constraints, but also to begin adoption of continuous improvement principles for which other mature manufacturing industries are known.

2.3 Biopharmaceutical Manufacturing

Although biopharmaceutical manufacturing, a subset of biomanufacturing, shares some characteristics with certain other liquid and solid manufacturing processes such as fermenting yeast and oil refining, it also includes a set of both unique and relatively immature processes, especially as compared to traditional pharmaceutical manufacturing.

Because biopharmaceutical manufacturing is still a somewhat novel process at large scales, an attempt will be made to describe the primary steps here. In reality, a variety of manufacturing processes for engineered recombinant proteins are in use or under development, including cellular processes and products derived from transgenic organisms such as goats, chickens, and plants. This description focuses on the cellular manufacturing platforms using mammalian (i.e. CHO) or bacterial (i.e. E. coli) cells, which are by far the most common model for producing biopharmaceuticals.

The biopharmaceutical manufacturing process begins with a small vial taken from a set of cells engineered to produce the desired product, also known as the cell bank. The contents of the vial are then placed into the cell culture process, where the environmental parameters and nutrient concentrations are manipulated to encourage a desirable rate of cell replication. During cell culture, an optimum level of parameters including acidity, oxygen level, carbon dioxide level, and nutrient presence are maintained in a bioreactor. As a critical mass of cells is cultured, the role of the cell changes from cell reproduction to protein production. This shift can be induced by changing the environmental conditions in the bioreactor.

The cells produce proteins until a desired amount of protein per unit volume, or titer, has been achieved. At this point, the process focus again shifts, and it now emphasizes protein isolation and purification. Newly generated therapeutic proteins share the bioreactor volume with cells, cell waste, nutrients, and a variety of other compounds from which they must be separated. The first major separation of proteins from cells is generally referred to as harvest, and the remainder of the separation process is called purification. Harvest can be performed in a centrifuge or filter that

Process Step	Description	Equipment	Desired Outcome
Cell Culture	Generate cells	Bioreactor	Critical mass of protein-producing cells
Production	Produce desired protein	Bioreactor	Target protein concentration
Homogenization (bacteria only)	Break cell membranes to expose protein	Homogenizer	Ruptured cells
Harvest	Separate protein from cell matter	Centrifuge	Proteins separated from cell matter
Purification	Separate protein from other impurities	Chromatography columns, filters	Purified protein in solution

Table 2.2: Major steps in the drug substance portion of biomanufacturing

separates the cells from the proteins. Afterward, the purification steps tend to be a series of chromatography operations, which involve passing the process fluid through columns packed with beads of resin that continue to isolate the desired proteins from undesirable compounds such as aggregated proteins, undesired proteins from the host cell population, and particulates. Then the process fluid is pumped through final filters to remove microparticles and viruses. The result of this process is a very pure, concentrated, protein solution called drug substance. The major steps leading to drug substance are outlined in Table 2.2.

Now that the protein is isolated, the next step is formulation, or adding other compounds to stabilize the protein or improve therapeutic characteristics such as residence time in a patient. Formulation may be followed by another filtration step. Finally, the drug product vials or syringes are filled, labeled, and packaged to be transported through a "cold chain," or temperature-controlled supply chain, to a location where they can be administered to a patient.

It is important to note that most of the biomanufacturing process often occurs in batches, where each step is conducted independently of any other. Furthermore, there are various stages during the process, such as after completing a batch of drug substance, where the product may be subjected to a combination of freezing, storage,

Process Step	Description	Equipment	Desired Outcome
Formulation	Add compounds to stabilize and adjust potency	N/A	Potent and effective biopharmaceutical
Fill/Finish	Segregate into separate doses (freeze if necessary)	Filling machine, freeze dryer	Properly dosed and frozen vials
Package/Seal	Seal vials, boxes, and label	Capping and labeling machine	Properly labeled and sealed containers
Cold Chain	Maintain temperature during transport	Insulated packaging, refrigerated vehicles	Effective therapy delivered to patient

Table 2.3: Major steps in the drug product portion of biomanufacturing

and transportation as needed. While these descriptions comprise most of the major biomanufacturing stages involved in directly producing and delivering the therapeutic protein, there are also many steps involved in preparing raw materials, managing utilities, quality control, and so on.

In conclusion, biomanufacturing is currently composed of a series of often discrete steps from cell culture to purification to final formulation and packaging. For a more detailed, yet introductory, explanation of biomanufacturing, the author recommends *Manufacturing of Pharmaceutical Proteins: from technology to economy* by Stefan Behme. Even though biomanufacturing as described in Behme's work is a validated process for protein production, these processes are constantly undergoing development and improvement in a variety of areas, including improved raw material usage, higher titers and concentrations, faster cycle times, increased continuous processing, and more effective process analysis and control.

2.4 Process Analytical Technology

Process analysis can be a broadly defined concept, and we will define it as equipment and instrumentation employed in analyzing an attribute or set of attributes of a flowing liquid or solid process. Typically, these process analyzers use one or more of a

variety of physical principles to measure a quantity (i.e. temperature), and then send a signal of some form to an interface where it can be understood (i.e. a reading in degrees Celsius) and acted upon by a human or automated operator. We will consider such analyzer-based control an essential part of any Process Analytical Technology (PAT) system. The system presented in the case study has a very simple "on/off" feedback control mechanism, while other systems might employ more sophisticated control schemes.

As in most industrial processing facilities, biomanufacturing facilities currently contain a variety of quality laboratories, process instrumentation, and other systems to ensure the manufacture and delivery of a quality product to the end user. However, the pharmaceutical industry (including the biopharmaceutical industry), has historically been slower than other industries to adopt new technologies and processes that result in higher quality products and more efficient manufacturing.

2.4.1 Regulatory Initiatives

At least one reason for this slow adoption was due to perceived regulatory uncertainty, so the FDA chartered an initiative called "Pharmaceutical cGMPs for the 21st Century: A Risk-Based Approach" in 2002, which was intended to encourage innovation within the pharmaceutical industry in hopes of achieving higher quality at lower costs. Two years later, a sub-initiative was launched called Process Analytical Technology (PAT), which was encapsulated in a document entitled "Guidance for Industry PAT—A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance." It defines PAT as "a system for designing, analyzing, and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality."

Understanding that a development effort is only sustainable if its implementation provides measurable benefit, the FDA enumerated areas where PAT is likely to provide returns, which are shown in Figure 2-2.[13] Then, in 2008, the International Conference on Harmonisation of Technical Requirements for Registration of Phar-

maceuticals for Human Use (ICH) released guideline Q8 for pharmaceutical development. This guideline formally introduced the concept of quality by design (QbD) to the pharmaceutical industry and enumerated PAT as a central tenet of pharmaceutical manufacturing. However, ICH Q8 altered the concepts of quality by establishing QbD as a lifecycle-oriented development framework with design of experiments, PAT, process knowledge acquisition, and risk management as its main components.[14]

- Reducing production cycle times by using on-, in-, and/or at-line measurements and controls
- Preventing rejects, scrap, and re-processing
- Real time release
- Increasing automation to improve operator safety and reduce human errors
- Improving energy and material use and increasing capacity
- Facilitating continuous processing to improve efficiency and manage variability

Figure 2-2: Expected benefits of PAT according to the FDA

Since the debut of pharmaceutical PAT in 2004 and its later association with QbD in 2008, PAT has understandably become a topic of interest throughout the industry. Matthew gives an accessible explanation of the relationship between QbD and PAT, while noting that regulatory initiatives for QbD and PAT were initially developed with traditional pharmaceuticals in mind. She also estimates the economic benefits of deploying QbD in the biopharmaceutical industry, substantiating the notion that QbD and PAT can deliver a cost effective impact to adopters.[15] Rathore and Winkle indicate that the Office of Biotechnology Products (OBP) within the FDA and similar departments at other global regulatory organizations are now responsible for encouraging QbD and PAT adoption within the biopharmaceutical industry. Furthermore, they specify that two of the greatest challenges faced by OBP and industry in the future are utilizing a common terminology and ensuring personnel from all organizations are appropriately trained to handle the imminent changes.[16]

In spite of these challenges, the regulatory agencies involved in defining and supporting the adoption of PAT have clearly injected interest and development in the area. This heightened interest has galvanized the development of many new technologies.

2.4.2 Technical Developments

One of the difficulties of discussing the topic of Process Analytical Technology in biomanufacturing is that the list of possible systems and ideas is vast. This could partly be due to the increased scrutiny given to PAT in recent years, and it also may lend credibility to the notion that many systems for measuring biological attributes continue to be insufficient for characterizing the key attributes of complex biopharmaceutical products. In order to provide some coherence to development efforts, several attempts have recently been made to review and categorize PAT developments for biopharmaceuticals.

One review, by Pitkänen et al. attempts to list "the state of the art in on-line bioprocess monitoring." Their review emphasizes recent inventions and innovations that are targeted exclusively for use on the bioprocess line. They also categorize systems by their measurement principle. The list is quite broad, but the systems are not necessarily PAT in their truest sense due to a lack of control systems in many instances. However, the review provides a look at the variety of analyzers on the market, which is an important component of any PAT system.[17]

A second, thorough, review is given by Rathore and others focusing on the topic of chemometrics in bioprocessing. The authors briefly explore the similarities between systems used in traditional chemical processing, traditional pharmaceutical processing, and applications in bioprocessing. By definition, chemometrics covers chemical analyzers such as spectroscopy, spectrometry, and chromatography, which accounts for a large part of the available biomanufacturing analysis systems. In this overview, the authors also emphasize the need to make effective use of increasing amounts of PAT-generated data.[18]

Känsäkoski et al. give an in-depth treatment of biometrics, or the use of biologically-

oriented measurement principles, in their review of areas where PAT needs to be developed. This review focuses on the cell culture and protein production phases of biomanufacturing, which is where biometric PAT applications would have the most impact. This is because the cell culture and protein production phases in the bioreactor have a longer operational time frame and a process fluid with high concentrations of biological species.[19]

Junker and Wang agree that appropriately applying PAT information from existing technologies can aid in bioprocess control, but they also encourage the continued development of new technologies that embody the principles of simplicity and robustness championed by Daniel I.C. Wang, Institute Professor at the Massachusetts Institute of Technology, whose pioneering achievements in computer-controlled fermentation methods laid the groundwork for pharmaceutical PAT.[20] Clearly, there is no shortage of opportunities both to incorporate existing technologies and develop new technologies for implementation as PAT systems.

The abundance of technology available and in development for use as PAT systems poses a conundrum as to how it can be effectively deployed in biopharmaceutical manufacturing. To aid in this effort, Garber outlines two essential business processes for creating and managing a PAT program within an organization. First, the PAT implementation process outlines criteria and decision-making around screening opportunities, identifying areas for potential PAT implementation, evaluating impact, and initiating the project to implement. The second essential process involves sustaining a PAT program once it is in place through a regular reporting structure. These discussions illuminate the fact that any innovative biomanufacturer seeking to embrace or improve PAT needs to lend momentum to such efforts through appropriate business processes and organizational structure.[21]

With an appropriate implementation program in place, one of the more intriguing prospects for PAT—also identified in Figure 2-2—is the facilitation of continuous processing. Warikoo et al. present a system for the continuous production of biopharmaceutical APIs, which is notably enabled by the use of a PAT system based on UV absorbance. As they indicate, the promise of continuous processing rests primarily on

its ability to reduce costs if effectively deployed, as has been demonstrated in various other industries.[22] While the method presented is not an "end to end" continuous biopharmaceutical production concept—in contrast to the concept demonstrated by the Novartis-MIT Center for Continuous Manufacturing[23] for small molecules—it is a promising possibility for overcoming a significant barrier to integrating the cell culture and purification steps of drug substance biomanufacturing.

2.5 Differences Between This and Other Research

The first difference between this and other research is that we present a method for approaching PAT implementation in an organization that emphasizes the entire analytical capability of the biomanufacturing plant, including in-process analyzers and off-line analyzers in all facility labs including quality control, in-process testing, and microbiological testing. Most research to date approaches PAT as a way to improve process control on a unit operation by unit operation basis, with little attention devoted to how appropriate use of PAT goes beyond a single unit operation toward facilitating continuous biomanufacturing or real-time release. While the scope of this research is limited to the portion of biomanufacturing that takes place in a facility devoted entirely to drug substance production, it provides evidence that PAT is at a sufficiently mature stage to provide tangible benefits. Gains from PAT can be sought by integrating improved raw material quality control, in-process control and release testing from raw materials to patient delivery.

The second difference is that we present a novel PAT system for analysis and control of the first clarifying chromatography step of biopharmaceutical manufacturing using fast detection of low levels of aggregate proteins at a manufacturing scale. This method couples static light scattering with UV spectroscopy. Previous work has also demonstrated improved chromatography control at manufacturing scales using a high pressure liquid chromatography (HPLC) analytical system interfacing with the process to a similar end.[24,25] The relative merits of the novel PAT system we present compared to other systems are discussed.

Chapter 3

Identifying, Evaluating, and Implementing Effective PAT Solutions

3.1 PAT Strategy

In order to develop a PAT strategy for the operational organization of a biopharmaceutical manufacturer, it is critical to clearly convey the key rationale for its deployment. A simple statement governing PAT strategy, shown in Figure 3-1, can be constructed by combining aspects of the definition, examples, and benefits of PAT.

> PAT manages product risk throughout the pharmaceutical manufacturing value chain through the innovative, effective acquisition and application of data

Figure 3-1: PAT strategy statement

Ultimately, PAT is about using data to manage risk during manufacturing. The data comes from validated and documented sources such as process analyzers and less rigorously documented sources such as human observation. The acquired data is then applied to manage risk through automated and human feedback mechanisms.

Therefore, a robust biopharmaceutical manufacturing process is a process that can accommodate the consequential and inconsequential variability inherent in manufacturing while generating an effective product with acceptably low risk of failure. For this reason, PAT is as much about understanding what attributes of the product and process are consequential as it is about developing technology to quantify those attributes.

3.2 PAT Opportunity Identification

As with most manufacturing facilities, biopharmaceutical manufacturing plants have a variety of areas and laboratories devoted to diverse tasks such as production, receiving raw materials, quality control, and shipping product. Each of these areas has a set of employees, processes, systems, and technologies that have been selected based on their capability to supply sufficient product to meet patient needs. These employees, processes, and systems are responsible for acquiring and applying data to ensure that the product is of an acceptable quality. Much of the previous literature around PAT has focused on the idea that opportunities for PAT can be identified by surveying the biomanufacturing process and screening the various unit operations for improvement opportunities. An illustration of this mindset is given in Figure 3-2. Clearly, this approach can yield benefits, but the maturity of biopharmaceutical manufacturing is reaching a point where a broader approach grounded in mitigating risk through the effective use of data is warranted.

Most biomanufacturing operations currently collect significant amounts of data. The current approach to verifying biopharmaceutical product quality is often a combination of ensuring that the process parameters measured during production remain within a specified range and verifying that the product meets specified criteria after being subjected to a battery of offline assays at various stages during the process. For this reason, current data sources include both online process analyzers and offline assays. In order to illustrate the type of testing and inspection that goes on in a biopharmaceutical manufacturing plant, Table 3.1 enumerates examples of various

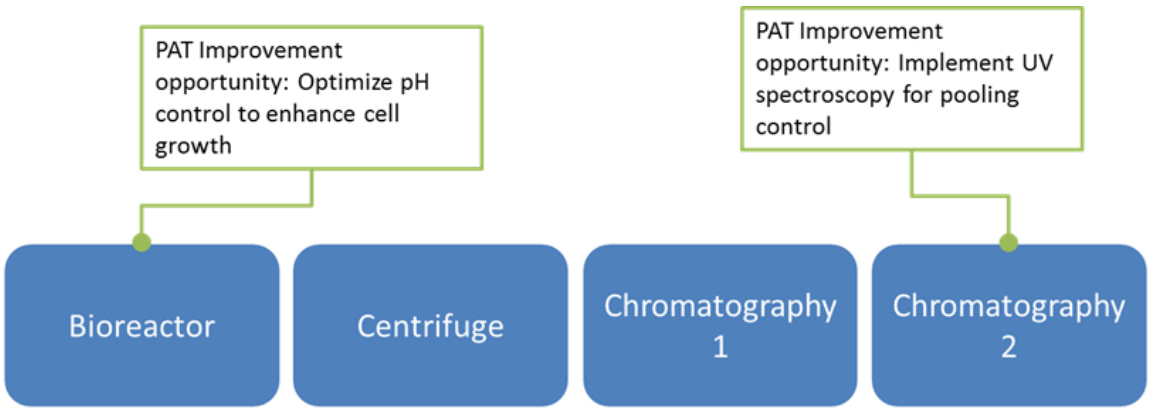

Figure 3-2: Unit operation-focused PAT opportunity identification

offline tests, or assays.

There are many other offline assays in addition to those listed in Table 3.1, and it is clear from the descriptions that the assays inspect characteristics of the protein itself, the protein solution, and impurities that may be present. Therefore, the numerous offline assays should also be an important contributor to any PAT strategy as they are ultimately part of the analytical capability of the plant. Both the data collected online in manufacturing and offline in the laboratories contribute to the overall picture of the risk profile of the process and product, so both sources should be utilized and developed to improve analysis and risk management.

Assay Type	**Description of Purpose(s)**
Size exclusion chromatography (SEC)	High molecular weight species (or average molecular weight)
UV spectroscopy	Protein concentration
Peptide Map	Confirm peptide makeup of (polypeptide) protein
Polymerase chain reaction (PCR)	Detect nucleic acids associated with impurities
Enzyme-linked immunosorbent assay (ELISA)	Protein binds to desired target to help confirm therapeutic efficacy
Visible inspection	Ensure that no visible defects are present in liquid
Kinetic limulus amebocyte lysate (Kinetic LAL)	Detect presence of bacterial endotoxins
Trypan Blue cell count	Count number of viable cells

Table 3.1: Examples of common offline assays

A central tenet of this thesis is that offline assays are another critical dimension for any PAT identification effort, adding to conventional, unit operation-focused methods of PAT opportunity identification. These two dimensions of a PAT strategy, shown in Figure 3-3, suggest a convergence of improving the manufacturing process with improving offline assays to establish a new generation of biomanufacturing plants that delivery higher quality products to patients.

This holistic approach also aims to highlight opportunities that exist for implementing continuous processing and real-time release in the hope that these objectives would increase the efficiency of biomanufacturing. In fact, continuous processing will increase the number of opportunities to use real-time control and modeling systems to manage the risk of the operating facility, thus expanding the PAT opportunities available to be explored. As long as PAT improvements are limited to a single unit operation, the opportunities to control the process will only be possible within the scope of each individual unit batch.

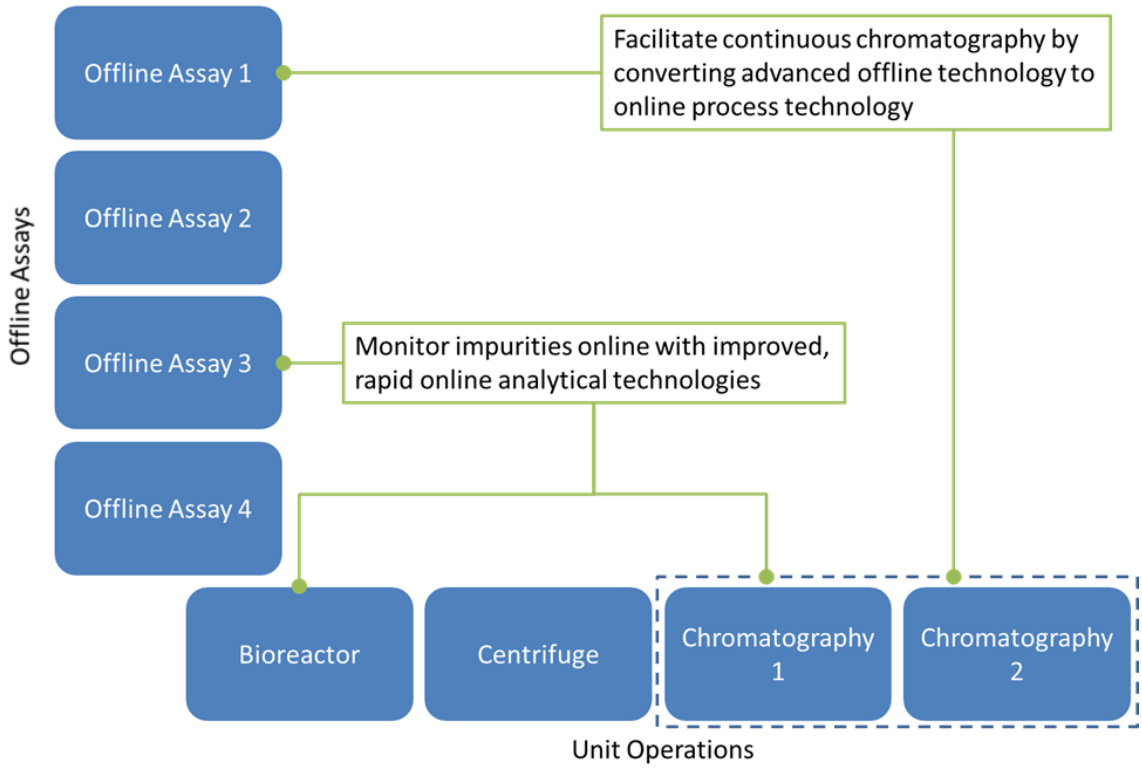

Figure 3-3: Two-dimensional strategy for PAT opportunity identification

This convergence also suggests that PAT solutions will not only come from developers of technologies with expertise in process analyzers, but also from developers with expertise in offline assays as they make improvements to their product offerings. In a general sense regarding PAT, offline assays can often be improved in many ways such as increasing measurement speed, reducing complexity, and improving process integration. Process-oriented technologies can often be improved by increasing sensitivity, enhancing reliability, and measuring new attributes of biological importance. This leads to the conclusion that some PAT solutions could improve control of the manufacturing process while simultaneously reducing the need for offline testing. Additionally, improvements to information technology infrastructure and control systems will facilitate this convergence of technological advancement.

In Figures 3-2 and 3-3, illustrations highlight the difference between approaching PAT from a perspective exclusive only to unit operation improvements and from a perspective that includes offline assay capabilities. However, these figures may understate the possibilities of using this approach to evaluate other strategic opportunities, such as raw material quality offline assays, the interaction between drug substance and drug product operations, stability testing, and so on. Therefore, this framework should be used to view the biopharmaceutical manufacturing facility as a single, complex, unit operation. This will provide new insight into how improvements can be made to the overall production process, and not just its constituent operational parts. Even in cases such as raw materials, combining batch process steps into continuous flow, and stability testing, the two-dimensional framework can be applied, resulting in both process technologies and offline tests contributing to opportunities for novel, effective PAT solutions.

In short, we propose a holistic approach to identifying PAT opportunities that includes evaluating opportunities both from the manufacturing process and offline analytical testing. This approach should serve as a complement to previously published frameworks based on evaluating single batch unit operations to maximize the effectiveness of implementing PAT in biopharmaceutical operations. All PAT implementation opportunities should be carefully evaluated to ensure that they are valu-

able, given that a holistic approach does not guarantee that all identified opportunities will provide a benefit. Implementing PAT, continuous processing, real-time release, and other technical advances could prove to be more cumbersome than beneficial if conceived and executed ineffectively. Therefore, in order to effectively evaluate those PAT opportunities that create value, a set of applicable criteria must be established.

3.3 Evaluating PAT Opportunities

An ideal biomanufacturing facility would be able to reliably produce therapeutics that adhere to quality specifications and ensure effective treatment of patients. All of the biopharmaceutical's characteristics that have a measurable physiological impact on the patient (including physiologically active impurities such as adventitious agents) would be measured and controlled. The majority of control would take place in real time, eliminating the need for excessive inspection, scrap, or rework post-processing.[13] In addition, the facility would maintain a continuous improvement process, enabling the improvement of cycle time, yield, and quality from acquiring and applying data. Not only would this knowledge be applied to improve a single facility, but each successive generation of facilities would improve on the prior generation. In this way, the biopharmaceutical manufacturing facility would effectively manage product risk.

Therefore, any PAT opportunity should be measured on its ability to deliver progress toward an ideal biomanufacturing facility given the time and cost required to develop it. In this thesis, we propose five categories to evaluate the costs and benefits provided by a PAT system, which are shown in Figure 3-4. These five metrics are one possible way to categorize the potential PAT benefits stipulated by the FDA as referenced in Chapter 2. The use of these categories is also influenced by the quality management principles championed by W. Edwards Deming and Joseph Juran and the concepts of the efficient plant set forth by Eliyahu Goldratt. These metrics can be viewed as a means to quantify the gain associated with any PAT-related implementation, and can conversely be viewed as a way to measure the reduced cost of poor quality of such an implementation. Just as the gain from implementing PAT

should be measured by evaluating its contribution to the four enumerated categories of benefits, the cost of implementing PAT must also be measured to ensure a net overall benefit. Key costs include the cost to develop, the time to develop, and the ongoing operational cost.

Quality
Producing product within specified ranges for a set of measurable attributes

Cycle Time
The time required to complete production of a given quantity of product

Yield
Quantity of product manufactured as a percentage of process inputs

Process Knowledge
Acquired knowledge that can be understood and applied for future improvements

Cost
The total cost of developing and implementing the opportunity

Figure 3-4: Criteria for evaluating PAT opportunities

In summary, the criteria of quality, cycle time, yield, process knowledge, and cost will serve as benchmarks for whether a PAT system is delivering a net benefit. With a framework in place to identify and evaluate PAT opportunities, we will now proceed to a case study of a novel PAT system. In the case study, the novel technology will be presented and considered in light of this framework.

3.4 Case Study: A Novel PAT System for Analyzing and Controlling Chromatography

3.4.1 Background

Protein aggregation during biopharmaceutical manufacturing is not uncommon, and is an area of focus for research because the presence of aggregates may cause problems

during delivery and use. As biopharmaceutical proteins have become more prevalent therapies, the discovered varieties and mechanisms of protein aggregation have also increased. In this work we will take the term protein aggregates, or aggregation, to mean any agglomeration of two or more protein monomers. A monomer will be defined as the smallest effective therapeutic molecule.

Cromwell et al. indicate that protein aggregation is undesirable because small, invisible aggregates may cause an adverse immune response in the patient, and large aggregates may cause delivery problems during injection. In either case, aggregation can occur when protein monomers denature, or unfold, exposing previously inaccessible covalent binding sites. Weaker, reversible, bonds can also form through electrostatic or dipole-dipole interactions associated with small changes in protein structure. In short, the size and complexity of protein molecules provide for a variety of pathways that can facilitate protein aggregation. Although we will treat protein aggregation here as unfavorable, some protein therapies are stable only when two protein molecules are covalently bound. Furthermore, Cromwell and her coauthors point out that many factors can influence and drive aggregation, including "temperature, protein concentration, pH...freezing, exposure to air...[and] mechanical stresses." Since many of these variables are inherent in the biopharmaceutical manufacturing process, aggregation can occur simply as a result of processing.[26]

Therefore, the ideal biopharmaceutical manufacturing product consists of a solution containing proteins only in monomer form, with minimal aggregate impurities. The mechanisms and sources of aggregation are still not well understood in detail, so they can be difficult to control. Consequently, a number of methods have been developed to address the issue during manufacturing, but biomanufacturers still desire simple, cost-effective methods to control aggregation.[27–29]

One segment of the biopharmaceutical manufacturing process that is a candidate for controlling aggregation is purification during the drug substance phase. Once the cell culture and harvest steps have been completed, the process fluid containing the proteins is pumped through a series of chromatography columns and filters for purification primarily by separation. The first column in the purification process

is generally a protein A, or capture, chromatography column. The purpose of this column is to induce binding of the therapeutic protein with the column resin, allowing much of the remaining material from upstream to pass through the column. However, capture chromatography does not appreciably reduce aggregation, since the column resin is designed to bind with an active site on the protein, even if that active site is on a protein that has aggregated with others. Therefore, the ideal purification step for removing aggregation is the next ionic exchange (IEX) column after the capture operation.

Typically, the nature of IEX chromatographic purification in biomanufacturing is such that the eluate, or output, from a given column is not of a constant purity during the course of operation. The proteins in the eluate can exit the column in varying concentrations over time, so an online measure of protein concentration, such as UV absorbance, has traditionally been used to determine when to stop elution to collect the column pool. In certain instances, such as when the therapeutic protein has very little propensity to aggregate, UV absorbance is sufficient for monitoring chromatography since the level of aggregation remains negligible during the operation. However, it has been shown that pooling by UV is suboptimal whenever the chromatography operation is being employed as a means to separate the product from impurities with similar UV absorbance characteristics like aggregates. This is because UV absorbance is a measure of concentration, but alone it does not directly measure the level of aggregated proteins.[24]

Online UV spectroscopy can measure protein concentration because aromatic amino acids—primarily tyrosine and tryptophan—absorb ultraviolet radiation. Therefore, the amount of absorbance of UV radiation in a sample volume can be an indicator of the number of amino acids in that volume, which provides a method to measure protein concentration. On the other hand, since UV only distinguishes the number of amino acids in a given unit volume, it does not provide a measure of whether the sample is composed of monomer proteins or aggregated proteins. To illustrate, x protein monomers in a sample would absorb basically the same amount of UV radiation as $x/2$ protein dimers because the same amount of amino acids are present in each. As

a result, UV absorbance is insufficient to determine the level of protein aggregation in a sample. Figure 3-5 shows an illustration of a traditional chromatography analysis and control system using UV spectroscopy.

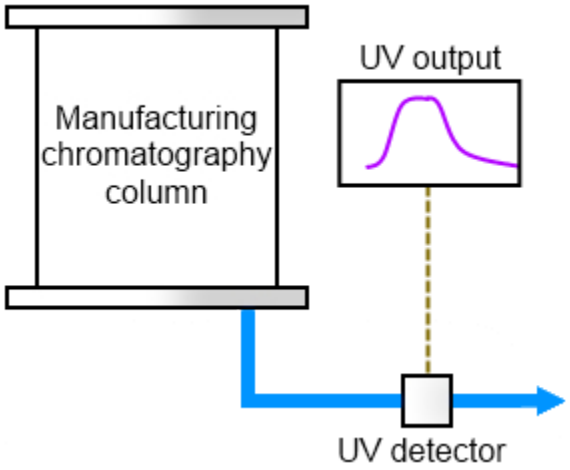

Figure 3-5: Illustration of chromatography analysis and control using UV

In this case study, we present a novel PAT system for analyzing and controlling chromatography during biopharmaceutical manufacturing. Specifically, the system focuses on chromatography unit operations that target the reduction of high molecular weight species (HMWS), such as the first IEX column. HMWS is a measure of the presence of particles with a molecular weight higher than the protein monomer, which includes protein aggregates. In addition, after a chromatography operation, we assume that the only high molecular weight species in the process stream are aggregated proteins, which is generally the case because capture chromatography will tend to retain only proteins—both aggregated and not aggregated—and will release any other high molecular weight particles. While the complete elimination of HMWS might be desirable, it is not practical because there is a trade-off between the amount of pure protein produced and the reduction of HMWS. We will see that since aggregated species begin entering the stream in small quantities early in the chromatography operation, complete elimination of HMWS would require a significant decrease in the yield of protein product. Therefore, we will consider the PAT system from the standpoint of controlling to a desired level of HMWS that is sufficiently low.

The opportunity for a novel PAT system was identified from improvements in an

offline analytical technology, static light scattering, in addition to improved technology in a class of industrial optical detectors, UV spectrophotometers. High-throughput, continuous methods for analyzing molecular weight of proteins in a laboratory chromatography column have been improving over the past several years. Since UV absorbance is correlated with protein concentration,[30] and since UV spectrophotometers are readily available, UV-oriented chromatography analysis and control has commonly been used to aid in meeting objectives such as reduced HMWS. However, instantaneous protein concentration in the process is not necessarily indicative of the amount of HMWS present in the chromatography pool. For this reason, a method for control that relies on a more direct method of measuring HMWS—or alternatively, protein purity—is desirable.

The proposed method for measuring the level of HMWS is assessing average molecular weight, which is commonly measured by combining UV spectroscopy with static light scattering on the outlet of a high pressure liquid chromatography (HPLC) column. This method is commonly used in laboratories, but the relationship between HMWS and average molecular weight depends on the composition of the sample, which will be discussed at length. The advent of UV spectrophotometers and static light scattering analyzers that can continuously measure protein characteristics in a flowing process stream, rather than being constrained to batch samples, has both increased the number of experiments that can be performed in a given period of time and increased the ability to measure how average molecular weight changes during the course of a chromatography elution. However, the instrumentation and analytical systems for SEC elutions are optimized for protein concentrations and process flow rates typical of a laboratory elution, which is on the order of 1 mL/min (flow rate) and 0.1 to 1 mg/mL (milligrams of protein per milliliter of solution). On the other hand, large-scale biomanufacturing purification process streams have flow rates in excess of 1 L/min and can see much higher concentrations. Therefore, these methods will not scale to the much higher flow rates and concentrations seen in commercial manufacturing without requiring the diversion of a dedicated mL/min flow from the higher flow rate process stream.

For this reason, attempts have been made to assess protein purity on-line in commercial manufacturing, particularly by increasing the throughput of a laboratory HPLC system and diverting a small flow rate stream to it.[25] An illustration of this setup is shown in Figure 3-6. While this approach is a valid way to assess purity of a protein stream on-line, it has the disadvantages of being a relatively complex system, requires a diverted flow stream for analysis, and is subject to residence time limitations due to holdup in the HPLC equipment. The primary advantages of the system are its accuracy, sensitivity, and similarity to traditional laboratory assays used to determine protein purity.

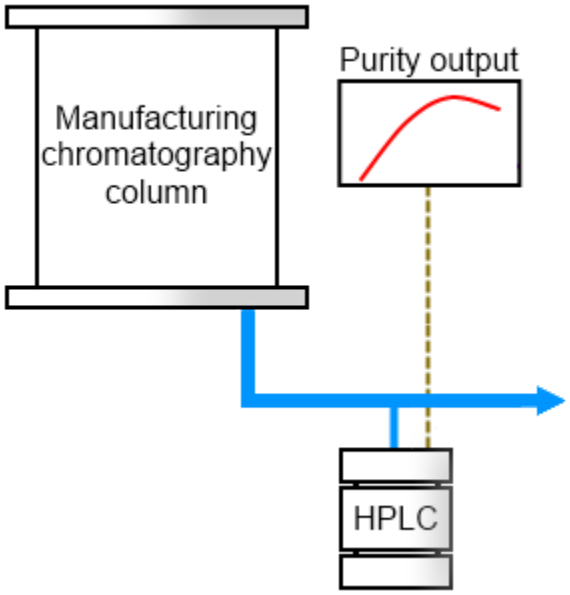

Figure 3-6: Illustration of chromatography analysis and control for aggregation using HPLC system

The novel PAT system proposed here takes a new approach by combining characteristics of each method. First, it employs UV as a measure of concentration, then it employs light scattering as a measure of particle size. Light scattering, when combined with UV detection, can provide an accurate measure of average molecular weight. In fact, this approach is not so different from on-line HPLC methods, since those methods often employ sensitive optical detection based on UV or light scattering principles to calculate purity. In a way, the novelty of this approach is the elimination of the additional HPLC columns, elimination of the pumping system, and transition of the

analyzers to the main process stream.

A key rationale behind this approach is that commercial-scale chromatography columns optimized for the reduction of high molecular weight species (HMWS) will clarify the process stream sufficiently for the average molecular weight of the proteins to be assessed without the need for an additional, analytical chromatographic separation. Additionally, light scattering and UV absorbance devices have reached sufficient size and throughput to handle the larger flows required for commercial manufacturing. This increased throughput does not necessarily increase the measurement volume, but it does allow for the measured volume (presumably a representative sample of the process) to be analyzed quickly and efficiently. For these optical devices, a key development that enabled their use in manufacturing was flow cells with short enough path lengths to measure undiluted process flow streams. Therefore, the proposed PAT system is one that employs a large-scale light scattering device and UV device.

The novel setup is shown in Figure 3-7. In order to further substantiate the concept, the large-scale system was also demonstrated using a laboratory scale light scattering device on a diverted low flow rate stream for analysis. Traditional manufacturing chromatography analysis and control methods also employ human or automated operators that monitor the output of a UV detector in order to stop elution before a predetermined level of concentration is reach. Since concentration in this case is taken to be an indirect indication of purity, this indicates that there is a tradeoff that can be optimized between product purity and step yield. The optimal elution can then be achieved by improving the PAT system used to analyze and control chromatography. For this reason, the proposed PAT system will also require modifications to the automation and control process to maximize benefits. These modifications include changes in human operator processes and algorithms used to control the chromatography operation. A core component of these changes is devising a suitable mathematical equation to calculate the average molecular weight online.

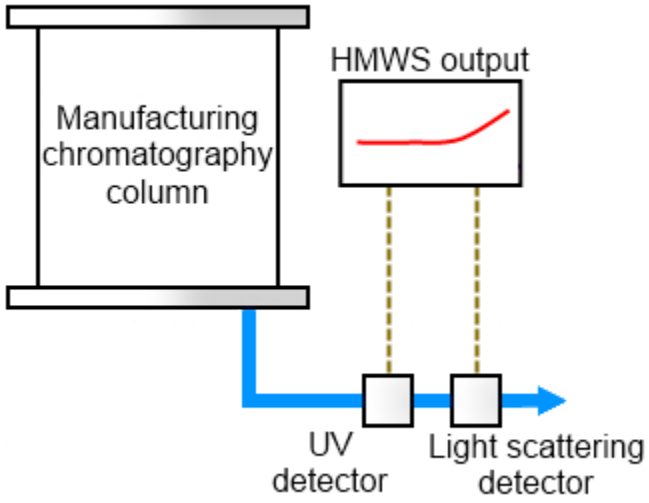

Figure 3-7: Illustration of chromatography analysis and control for aggregation using novel PAT system

3.4.2 Theory

The theoretical basis for the proposed PAT method of measuring average molecular weight online is that protein concentration can be measured using UV absorbance[30] and that the process stream at the outlet of the chromatography column can be modeled as a solution of particles that scatter light akin to other particles that are much smaller than the wavelength of the light that they are scattering. Dollinger et al. demonstrate that such a protein solution can be modeled using the Rayleigh-Gans-Debye approximation of the Mie solution to Maxwell's equations for light scattering as shown in Equation 3.1.

$$\frac{K\rho}{\overline{R}(\theta)} = \frac{1}{\overline{M}P(\theta)} + 2A_2\rho + O(\rho^2) + ... \tag{3.1}$$

K is an optical constant of the solution, ρ is the solute (protein) mass concentration, $R(\theta)$ is the average excess Rayleigh ratio, $P(\theta)$ is a scattering factor related to size and shape, and $\overline{M}$ is the average molecular weight.

This model is for the process stream, or protein solution, at an instantaneous point in time. It can also be thought of as an infinitesimally thick cross-section of the process fluid that is measured by the analyzers. The approximation is expanded

by powers of ρ, so A_2 represents the second virial coefficient in the expansion. We will presume at this point that orders of ρ greater than or equal to 2 are negligible for ease of computation. It can then be assumed that light scattering is independent of size and shape, so $P(\theta)$ approaches 1 because the protein is not sufficiently large compared to the wavelength of light emitted.

While the literature can be consulted for additional information on calculating, K and $R(\theta)$, it is worth recognizing that K contains constants related to the inherent properties of the light, solvent, and solute, while $R(\theta)$ can be explained as containing the variables for intensity of detected scattered light at a given angle relative to a baseline background scattered light intensity. We will also assume that the refractive index of the solvent, contained in K, remains constant.

Therefore, as Dollinger and his coauthors indicate, the equation to calculate average molecular weight can be reduced to Equation 3.2 based on the assumptions given. I_S is the output of a light scattering detector placed at 90° from the light source, A is the absorptivity of the protein, and UV is the absorbance of UV radiation as measured by the analyzer. The term for ρ from Equation 3.1 converts to UV and A because concentration is measured using UV spectroscopy and Beer-Lambert's law is assumed. The term for $R(\theta)$ converts to I_S if a single light scattering detector is placed at an angle from the light source. k'' is an instrument and angle-dependent constant, and A and k'' can also be combined into a protein and instrumentation dependent constant.

$$\overline{M} = \frac{I_S A}{k'' UV} \tag{3.2}$$

In short, the measurements of interest for determining average molecular weight are the absorbance of ultraviolet light—normally with a wavelength of 280–300 nm—by the process stream, and the intensity of scattered light—normally with a wavelength of 500–700 nm) at a 90 degree angle. The scattered light wavelength of 500–700 nm is desirable because the scattering of light by the proteins begins to deviate from the model as the wavelength deviates from this range. Furthermore, Equation 3.2

can be used independently for any angle of light scattering detection provided the constants are adjusted accordingly.[31]

Several assumptions are made in this simplification, namely that the protein of interest is very small compared to the wavelength of light scattered (i.e. 500–700nm). Since most commercial therapeutic proteins are monoclonal antibodies with a radius of less than 10 nm, and other therapeutic proteins are generally smaller than monoclonal antibodies, then this assumption holds for our purposes. The equation also assumes that the optical detectors are calibrated correctly and that the specific refractive index increment of the solute and absorptivity of the solution remain constant. Furthermore, this also assumes that the concentration is not so high that higher order terms of ρ impact the calculation.

These assumptions will serve for the purposes of demonstrating this novel system, and the consequences of these assumptions will be discussed in more detail. In this way, we see that the average molecular weight of proteins in a process stream is related to the amount of light scattered (as measured by the light scattering detector at a given angle) and the concentration of proteins in the solution (as measured by a UV spectrophotometer).

3.4.3 Materials and Methods

Four experimental runs were conducted on large-scale (30 cm diameter) IEX columns in a pilot plant facility. The flow rate for runs 2, 3, and 4, was held constant at a flow rate of approximately 2 L/min in the ¾" outlet line of the column. The flow rate for run 1 was 0.8 L/min. For online UV detection, an Optek® AF46 was used, and a customized Optek turbidimeter was employed for online light scattering detection (with detectors at 11° and 90°). For laboratory-scale replication of the data, a Wyatt DAWN HELEOS® II and an Agilent 1200 series UV-Vis detector were used. The customized Optek turbidimeter was placed directly on the 2 L/min process stream, while the Wyatt DAWN HELEOS® II was limited to a flow rate of 2 mL/min. The column packing and size was optimized to the protein of interest.

Laboratory assays of percent purity (and percent HMWS) were conducted by

SE-HPLC according to methods approved for use in commercial biopharmaceutical production. All instrumentation for the large-scale experiments was connected to a digital process automation system to test and prove control criteria and algorithms. The run charts displayed in the figures were based on online data sampled by the process automation system every 0.5 seconds. The one exception to this is the offline percent HMWS measurement, which was based off of samples extracted from the process once every 3 minutes. In order to facilitate visualization, the online data (UV, I_S, and $\overline{M}$) in the figures was resampled at the same 3 minute intervals. However, because the online data was sampled at time intervals significantly shorter than the scales presented, it is shown to be continuous.

Samples were extracted from the process at regular intervals to verify the percentage of HMWS in the process at a given elution time. These samples were taken by initiating a pump that diverted a small amount of the process to sample vials in a rotating fraction collector until they were filled to approximately 3 mL. At 1 mL/min, the 3 mL fill would take 3 minutes, and then the next sample vial was automatically rotated into place. We assumed that the % HMWS measurement of each sample was representative of the process at the midpoint of collection time. For calculations of % HMWS in the pool, we verified the volume of each sample and then calculated the accumulated % HMWS up to a given sample from the time of initiating the gradient elution by using the accumulation of individual measurements.

All experimental runs were performed with the same monoclonal antibody, but the antibodies for each run were generated by separate bioreactor purification runs. For the second experimental run, the cell culture conditions were sufficiently different to significantly change the HMWS profile during the run, so it is not included in the results section. Tabulated data from the experiments is included in the Supplemental Information section of Appendix A.

3.4.4 Results

The goal of the novel PAT system presented here is to offer a new approach to analysis and control of large-scale chromatography specifically with the intent of reducing the

level of aggregated protein species in biopharmaceutical manufacturing. The system is based on employing a UV detector and a light scattering detector near the outlet of the manufacturing column and using the analyzer outputs to monitor the average molecular weight of the process stream at a given point in time. This, as will be discussed, can be used to control the level of high molecular weight species (HMWS). The system is most notable for its speed and simplicity.

A system capable of online control must be able to output reliable online data. To illustrate a practical application of Equation 3.2, an illustration of the analyzer output and average molecular weight calculation of a typical experiment is given in Figure 3-8. For this and all subsequent figures, UV represents absorbance at 300 nm as a fraction of maximum absorbance recorded over the course of the experiment, in other words $UV = \frac{UV}{UV,maximum}$. Similarly, light scattering output values will be scattered light intensity at 90° as a fraction of its maximum, or $I_S = \frac{I_S}{I_S,maximum}$. The initial units of UV as output by the detector were OD (optical density), and the initial I_S units were in volts. Finally, protein molecular weight will be given as the fraction by which it exceeds the baseline monomer molecular weight, or $\overline{M} = \frac{\overline{M}}{\overline{M},monomer}$. The average molecular weight of therapeutic proteins tends to be in the range of 50–200 kilodaltons (kDa). Since the redefinition of the variables, UV, I_S, and $\overline{M}$, results in their becoming relative values, they will now also be unitless quantities.

As illustrated in Figure 3-8, the monomer protein elutes first, so the average molecular weight of the proteins in the process stream at any point in time is constant until a transition point when the molecular weight of the process stream begins to increase (in Figure 3-8, this occurs at about 1 hour). While the experiments published here were all performed with a single type of protein, other parallel experiments with other types of proteins indicated similar trends. This helps confirm the assumption that only pure monomer elutes early and larger species elute later. Correspondingly, UV and I_S are equal as long as the average molecular weight of the proteins equals the average molecular weight of the monomer. UV and I_S diverge when the average molecular weight of the proteins exceeds that of monomers. Therefore, by measuring UV absorbance and light scattering, a real-time measurement of instantaneous

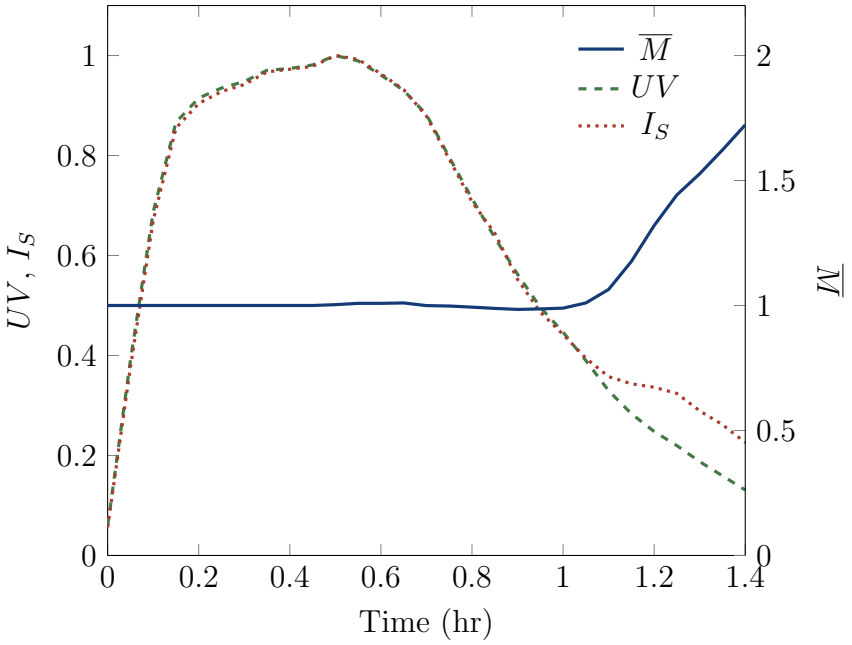

Figure 3-8: Example of overlaid UV, I_S, and $\overline{M}$ run charts during an experimental run

average molecular weight can be calculated.

This approach can be applied when the maximum UV absorbance and maximum scattered light properties of the elution are both known and unknown. If they are known, then k'' and A from Equation 3.2 can be calculated, and a run chart of the elution would look similar to Figure 3-8. However, in some cases, especially during process development, the expected absorbance and scattered light values are not known with much certainty, if at all. Furthermore, issues with instrument calibration and drift may cause changes in the measured maximum values from run to run. Using arbitrary k'' and A values would cause $\overline{M}$ not to be constant during monomer elution and the UV and I_S values would not match. One way that this problem can be addressed is by incorporating an algorithm for detecting the maximum UV and I_S values in real-time, and once the maximums are detected, immediately inputting the values into the molecular weight calculation at the time of detection. This would ensure that after the maximum values are detected, all future calculations during the run would be accurate. This is particularly helpful because the transition point when aggregated species begin eluting is a sufficient amount of time later than when

maximum UV and I_S values are recorded.

Flow rate effects and time alignment of analyzer outputs can be other important issues to consider. In systems similar to Figure 3-6, the low flow rate stream diverted to the analytical equipment can present an issue because the time it takes for a given unit of the process stream to be diverted and analyzed can delay the ability of an operator to take action. Similarly, using a laboratory-scale light scattering detector in an equivalent setup could cause a noticeable delay between the UV detection of a given unit of process stream flow and the corresponding scattered light detection due to dead volume in the line and the pump required to deliver the stream to the analyzer.

Therefore, this PAT system in concept presents a novel method for controlling pooling during chromatography because it allows for the elution to be stopped—and the batch to be collected—at a point in time that corresponds with a desired instantaneous average molecular weight of the process proteins. Normally, though, the percentage of high molecular weight species (HMWS) by mass in the collected pool is desired, but this cannot be calculated without knowing the composition and types of HMWS present in the process stream—be they dimers, trimers, or larger aggregates. Conversely, if the composition profile as a function of time is known, then the % HMWS in the pool can be calculated. If we assume, for the moment, that any increase in average molecular weight is due only to the introduction of dimer aggregates, or two bound monomers, then the instantaneous average molecular weight of the proteins will change linearly with the percent HMWS in the collected pool. As shown in Figure 3-9 for the same experimental run, $\overline{M}$ appears to correlate linearly with the percent HMWS in the pool over the time period shown, suggesting that for this time range the process stream is composed almost only of monomers and dimer aggregates. The HMWS value in the figure was calculated by using a validated offline method for such a measurement. As displayed, the detectable dimer aggregates begin entering the stream at approximately the 1 hour point. However as indicated in the figure, the novel PAT system is not quite as sensitive to low levels of aggregate species as analysis performed offline in a laboratory.

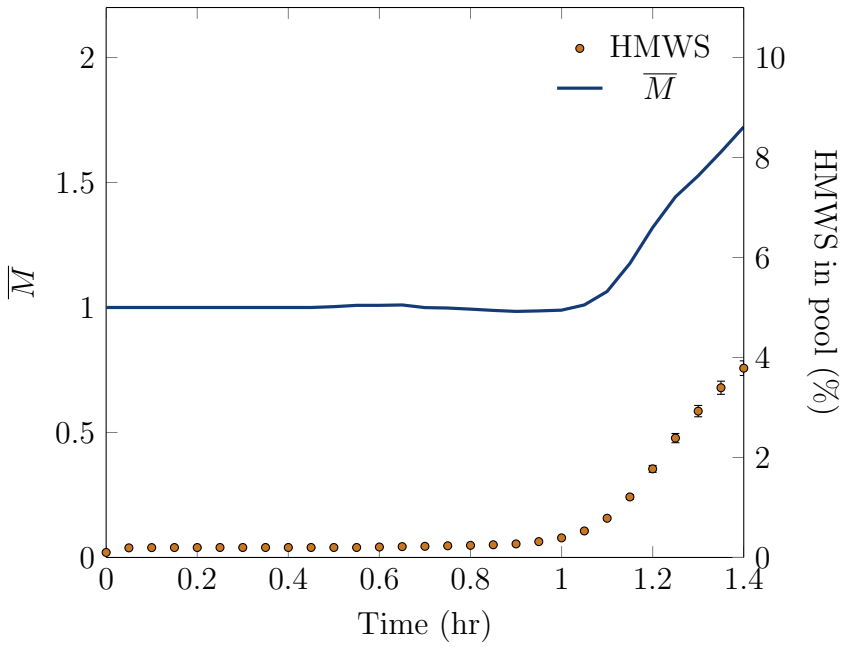

Figure 3-9: Example of $\overline{M}$ run chart and % HMWS in pool over time - error bars represent one standard deviation around the mean and may be obscured by the chart symbol

This linear relationship holds because the % HMWS in the pool is now directly proportional to the mass fraction of dimer in the pool at a given time, which is directly proportional to the instantaneous average molecular weight. At some point in the elution, the process stream contains larger aggregate proteins, so the linear relationship no longer holds. But in most cases, including cases reviewed for other therapeutic proteins, the optimal point for stopping elution and collecting the pool is before these much larger aggregates begin to appear. This is due to the optimum collection point being one that minimizes high molecular weight species without compromising process yield, so we will not examine the elution region containing detectable larger-than-dimer species. Rather, we will assume that elution is stopped sometime before a level of 3% HMWS is reached for this protein (i.e. about 1.5 hours in Figures 3-8 and 3-9).

Accordingly, Figure 3-10 shows the correlation between % HMWS in the pool and $\overline{M}$, where the former is plotted as a function of the latter. This data was gathered from 3 experimental runs under similar conditions. These data were also replicated

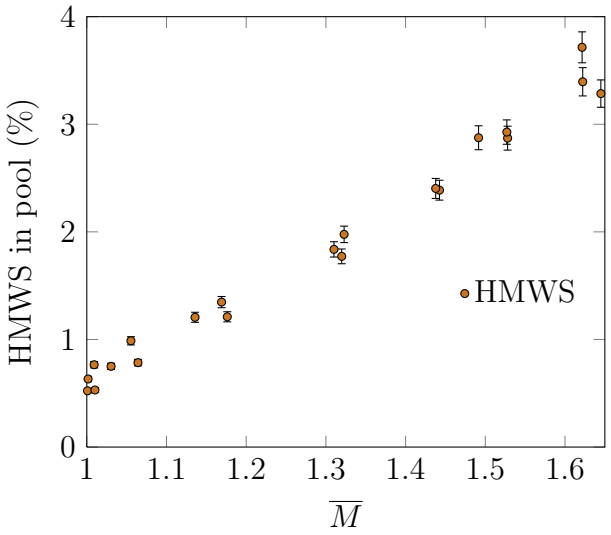

Figure 3-10: % HMWS in pool as a function of $\overline{M}$ - error bars represent one standard deviation around the mean

smaller scales, but this replication is not shown here. However, the results from those experiments were similar.

We see that $\overline{M}$ does not exceed 1 until the % HMWS in the collected pool exceeds 0.5%. This suggests an approximate detection limit of the current method as presented. For the % HMWS values greater than 0.5% (above the presumed detection limit), the linear R^2 for the data is 0.983. This lends credibility to the notion that for this specific application and range of % HMWS in the pool, $\overline{M}$ can be used to calculate the accumulated high molecular weight species in the pool because the system behaves only as if the process is composed of monomer protein and, later in the elution, dimer aggregates. Data reviewed at values greater than 3% HMWS in the pool indicate that, as expected, this correlation becomes nonlinear, indicating trimer and higher order aggregates begin eluting at approximately this time.

While 2 L/min can be considered manufacturing scale, some large biomanufacturing processes can exceed that flow rate. For processes that operate at an even larger scale, a few assumptions would need to be revisited and tested to ensure that this novel PAT system continues to provide an effective means of analyzing and controlling chromatography. First, the sensitivity and reliability of the detectors and light emission source are important aspects of optical measurements, and improvements in

these areas may improve the correlation of the molecular weight calculation and the % HMWS in the pool. Second, while the general method described will hold true for a variety of manufacturing operations, different column resins, pumps and other process equipment changes will likely have some impact on the data. Third, while the concentration used in these experiments is very high by most current industrial standards, additional increases in concentration will require revisiting the use of Beer-Lambert's law and a second order Rayleigh-Gans-Debye approximation to calculate the instantaneous molecular weight. Fourth, as previously mentioned, there are other methods for assessing process stream purity, and judgment is required when assessing whether the benefits of speed and simplicity outweigh the alternatives of precision and sensitivity. Finally, while the run to run variability appears to be low, more research is needed in establishing a method acceptable both to the bioprocessor, patient, and regulatory agencies.

In spite of these qualifications and areas where variability can be introduced, it bears restating that this method is useful because of its simplicity and speed. In addition, what seemed like rather significant assumptions in theory turned out to have a very low impact on the actual results. For this reason, scaling up flow rate and concentration could have minimal impact on the results presented here.

3.4.5 Limitations

The novel PAT system presented in this thesis does have some limitations. First, as previously mentioned, it is currently applicable only to chromatography systems where large particle reduction of dimer size or greater is an important objective of the targeted chromatography step. Because the system is based on principles of light scattering by particles, a process eluate that contains large amounts of scattering particles, which are not proteins, will scatter excess light and deviate from the proposed model. Therefore, this system assumes that the manufacturing chromatography column and any prior processing steps clarify the stream sufficiently to avoid such a scenario. Similarly, other particles may interfere with the use of a UV-based analysis and control system as well. The light scattering detection device, or turbidimeter,

used for our experiments was the joint development effort of the supplier and our team, so it is not yet widely available.

Second, there was an experimental run that yielded outlying data that was not presented with the results. In short, for that particular run the level of aggregated species was significantly lower than the relatively consistent levels displayed in the results. While the precise source is under investigation, evidence indicates that changes made to processing conditions upstream of the chromatography operation resulted in various downstream effects, including lower aggregation levels. Since this was a developmental run, such changes and excursions are not unusual. However, since the changes were significantly different from the other runs presented, the data was not included in our results. Before therapeutic proteins are produced commercially for patients, the biopharmaceutical manufacturing process is designed and engineered so that the risk of unexpected changes is minimized. This scenario was instructive as a means for testing the novel PAT system's sensitivity to excursions and process upsets.

Finally, this system has only been tested on a single chromatography step targeting the reduction of HMWS in the purification phase of drug substance manufacturing. As discussed in Chapter 2, biopharmaceuticals are generally less stable than other therapeutics, and this low stability extends to aggregation. Under certain conditions, including those optimized for long-term storage, proteins can preferentially aggregate over time depending on their particular characteristics. Therefore, any efforts to reduce HMWS during drug substance manufacturing may be negated by aggregation occurring later in the manufacturing and supply chain. Since biopharmaceutical companies keep relatively high inventories of product—and thus high product residence times in the value chain, it is important to realize that this novel PAT system would be just one component of a total aggregation control system.

In spite of these limitations, the novel PAT system is a beneficial addition to the analysis and control toolkit of both widely-used and recently developed chromatography analysis and control systems. In an effort to directly compare various aspects of the novel system against other systems, Table 3.2 has been included to show the advantages and disadvantages of each. While developing suitable analytical equipment

Description	Advantages	Disadvantages
Novel system (UV and light scattering)	Rapid analysis Simple system Directly measures $\overline{M}$ Relatively inexpensive	Assumes only monomer and dimer HMWS
Online UV (traditional)	Rapid analysis Simple system Inexpensive	No HMWS analysis
Online HPLC	Most sensitive to HMWS Analysis independent of composition	Slower analysis time Complex system Relatively expensive

Table 3.2: Advantages and disadvantages of novel PAT system relative to similar systems

and hardware is an important part of implementing a PAT system, careful attention must also be paid to developing the software algorithms, control system, and operating procedures as well. In reality, many biopharmaceutical manufacturing facilities use commercially available analysis and control systems customized to the extent that the vendor and manufacturer can agree upon. Furthermore, constraints on the number and skill level of operating personnel must also be considered. Finally, the entire PAT system must be able to bear the rigors of long-term, high-volume manufacturing while reliably controlling a process that delivers a safe and efficacious product to the patient, ensures the safety of the operating personnel, and minimizes the impact on the environment.

Wiring, input/output (I/O) hardware, and control scheme programming are important aspects of any PAT system, but an in-depth discussion will not be given here. Rather, we will focus on a few key considerations for PAT systems in biopharmaceutical manufacturing facilities. As discussed in Chapter 1, the recently issued

PAT guidance has galvanized an effort that is being more openly embraced within the biomanufacturing community. While many unit processes and operations, especially bioreactors, have made significant advances with PAT, the use of PAT on a manufacturing facility level has not been standardized within the industry. For this reason, different process analysis and control systems have varying degrees of "plug and play" characteristics, or the ability to be easily integrated into biopharmaceutical operations.

Because of the complexity of implementing new hardware, software, and control systems, the installation of new PAT systems must involve a review of what data is being transmitted from each analyzer and how that data should be used to measure the attribute of interest. For example, in the novel PAT system presented, both a UV spectrophotometer and light scattering analyzer are required to assess different characteristics of the process stream, which in turn, are synthesized to calculate average molecular weight. However, the biopharmaceutical manufacturer often has a choice as to whether they combine data and make calculations within the distributed control system (DCS) itself, or whether they do so in a separate processing device prior to sending the final data stream to the DCS for control purposes.

In existing facilities, the decision will be strongly influenced by the capabilities of the facility and personnel. However, in a new facility, there is often flexibility to choose the optimal solution based on the PAT evaluation criteria outlined in section 3.3. In the case of this PAT system, after interviews with various operations and automation experts, we resolved that the optimal scenario was to send the UV and light scattering data signals to the DCS system and use the DCS to make the molecular weight calculation. The simplified equation for calculating $\overline{M}$ greatly facilitates this application, and there were other applications where the separated signal outputs from the UV and light scattering analyzer could be useful (i.e. calculating total mass of protein processed or process stream turbidity). In any case, the average molecular weight data transmitted within the DCS system can then be used to control a pump or valves as necessary. Alternatively, a human operator actively monitor the relevant output with complementary operating procedures to facilitate good manufacturing

judgment.

The degree to which a PAT system should be automated is also an important consideration. Automation, when carefully planned and implemented, can enhance the quality of an operation, but there is often a tradeoff between the complexity, or risk, of an automated system and the benefit from automation. For example, in the case of the novel PAT system, if an operator is already closely monitoring a small set of other process attributes during the chromatography elution, then automating one pump or valve may be unnecessary and add no additional value. However, if a required sample preparation and analysis is sufficiently frequent and time consuming, then automation may help reduce the burden and stress on operators.

Operating procedures are also an extremely important aspect of the PAT system. Procedures should be developed in collaboration with the hardware, software, controls, and automation disciplines in order to ensure effective operation of the facility over the long-term. Ideally, procedures are sufficiently detailed to operate the facility, but not so detailed as to be cumbersome. Effective procedures and proper process discipline will ensure that the plant is operated effectively and safely.

The need for development of effective experimentation coincides with another important need to develop personnel capable of using sound judgment when making decisions based on PAT-derived data. The increase in the number of PAT systems in biopharmaceutical manufacturing facilities will require management, operational, and technical staff to become more adept at working with an evolving set of process protocols. These personnel will also need to ensure that PAT systems developed in the future are not only technologically advanced, but that they can be effectively operated in such a way that optimally manages product risk.

3.5 Designing PAT for risk management and for the end user

In many situations, biopharmaceutical manufacturers have operated plants that have two distinct areas and organizations, both of which are critical to the effective and safe manufacture of biopharmaceuticals: the laboratory and the manufacturing floor. In some cases, even different laboratories and manufacturing areas can develop distinct cultures, but for this discussion we will primarily consider the difference between labs and operating areas. As indicated in Chapter 1, the laboratory and manufacturing floor are the two primary dimensions from which PAT system concepts should be generated, and a general understanding of how PAT can unify these areas and the challenges involved is merited.

For laboratory-based tasks, the analytical systems are generally designed and engineered to measure a specific attribute or attributes with a high sensitivity and accuracy. For process-oriented technologies, the emphasis tends to shift toward robustness to a manufacturing environment, reliability, and rapid analysis. When developing a PAT system, it is crucial that individuals with expertise in both areas are involved in development, because the ideal PAT system encompasses characteristics from both areas. Furthermore, a focus on the needs of the intended PAT application is paramount. Understanding how frequently the system should measure an attribute, how robust the hardware needs to be, who will be responsible for operating and maintaining the system, and how it interfaces with the facility control system will help determine the optimal PAT system design. A methodical approach of this nature will help minimize suboptimal outcomes where development emphasizes a narrow set of criteria that results in an unusable PAT solution.

The novel system presented here was first evaluated in a laboratory before attempts were made to scale up the system for large-scale operations. It is important to note that, in many ways, the laboratory-scale system and large-scale system are similar in concept and purpose, but a simple proportional increase of the laboratory system to manufacturing scale would have rendered the system unusable. For

this reason, it is critical that the needs of the end user be considered at every stage of the development process. For example, the light scattering analyzer used at the laboratory scale was optimized for use with laboratory hardware, control systems, and flow rates. Using the same device in manufacturing would have constrained the system to a diverted flow stream and reduced the benefit it provides. Therefore, we researched and developed the manufacturing-scale turbidimeter that had the capabilities we needed, and it was optimized for the needs of a large-scale, biopharmaceutical manufacturing environment.

Conversely, this approach may jeopardize the very results that are obtained at small scales, so rapid, cost-effective experimentation must be employed to optimize key attributes of the system as it moves through development. For example, light scattering analyzers often measure scattered light at multiple angles to improve the accuracy of the molecular weight calculation. However, in the case of the PAT system presented here, the high concentration of protein in the process stream relative to concentrations typically seen by light scattering analyzers helped determine the ideal number of measuring angles that would most effectively determine average molecular weight. By designing and executing a series of simple, fast experiments, we were able to reach an informed decision regarding the optimal state of this attribute .

As PAT systems are deployed in biopharmaceutical manufacturing operations, additional questions remain from an organizational perspective regarding how to deploy personnel within the manufacturing, quality, and technical subdivisions of the operational organization structure. While a variety of different organizational regimes are in place throughout industry, a key trade-off that deserves discussion is balancing the role of the quality organization as an auditor, the role of manufacturing as the efficient producer, and the role of research and engineering as the technology developer. Some organizational separation between the groups facilitates the development of functional expertise in specific areas and helps employees feel a sense of progression as they acquire and improve capabilities. However, excessive division, or "siloing," can result in restricted flows of information that are necessary for the organization to effectively achieve its goals. Notably, a quality organization is required by regulatory

organizations, so the degree of integration must adhere to these regulations. As far as PAT systems are concerned, it is imperative that quality, manufacturing, and technical personnel interact in a way that ensures that feedback, learning, and continuous improvement are commonplace.

Chapter 4

Areas of further research and development

In Chapter 2 we reviewed literature regarding process analytical technologies that, for the most part, are either currently in use or commercially available. Many of these technologies are continually being refined and have great potential for bringing significant benefits to biopharmaceutical manufacturing in the future. There are also many technologies that have been developed in recent years, have been identified as potential PAT applications, or could become key components of future biopharmaceutical manufacturing PAT systems. Because most of these technologies are recent, the horizon for implementation as PAT tools may be a few years away, but the purpose of this investigation is to shed light on some promising opportunities.

4.1 Advanced optical technology

An area of ongoing development for future PAT systems is optical analysis. Many optical technologies have been used in biopharmaceutical operations in some form or another for years, but generally they have not been widely employed as PAT systems, with the exception of UV spectroscopy. The novel PAT method described in Chapter 3 is one example of an advanced optical technology, because even relatively simple systems employing UV and light scattering detectors have not been previously used

to analyze and control large-scale chromatography.

The literature describing optical systems is robust, and there are several key areas of development regarding the use of optical technologies in biomanufacturing. First, PAT systems employing Near Infrared Spectroscopy (NIRS) are a promising opportunity for improving biopharmaceutical manufacturing. Measuring absorbance in the near infrared region of the electromagnetic spectrum is challenging in a manufacturing environment, especially for online applications. Scarff, et al. note that sufficient progress has been made both in analytical and computational technology to ensure that NIRS could be used in the future as a reliable, online analytical system. While the signal from a NIRS system can be more difficult to resolve into meaningful data than, a UV absorbance signal, for example, measuring absorbance of near-infrared radiation can be a very effective method for rapidly assessing the amount of nutrients and wastes in a cell culture.[32]

In addition to NIRS, Raman spectroscopy is a promising advanced optical technology that is a candidate for evaluating the multiple components contained in cell culture media. As indicated by Li and coauthors Raman spectroscopy systems have a unique advantage over other technologies because water is a weak Raman scattering compound. Also, Raman spectroscopy methods generally require no special or lengthy sample preparation regimes. The system developed by the authors demonstrates the application of Raman spectroscopy to analyzing cell culture media used in CHO-based bioreactors, which is another area where PAT systems could satisfy a biopharmaceutical manufacturing need.[33]

Clearly, advanced optical technologies offer promising advantages that are well-suited to PAT systems, such as rapid analysis and control of multiple components in a process. While the rate of adoption of advanced optical technologies remains low at manufacturing scales, recent improvements in signal processing, analysis, and computation promise to improve the prospects of such devices for inclusion in biopharmaceutical manufacturing facilities.

4.2 Microscale and nanoscale devices

A microscale device employs the space-saving attributes characteristic of microchips in order to fit all of the components of an integrated system in a miniaturized footprint. At times, microscale devices contain all of the requisite materials required to perform one or several laboratory assays (also referred to as "lab-on-a-chip"). While there is some overlap between the categories of microscale devices and optical analytical systems (i.e. hybrid microscale optical devices), the purpose of this section is to focus on PAT developments within the microscale category. Such devices have existed for decades, but many significant developments have recently been made in the area pertaining to biopharmaceutical manufacturing and biological analysis.

Love offers his perspective on bioanalytical technologies and how—when properly designed—they can improve analysis for biological applications. He describes the current state of bioanalytical microchips and their focus on scaling down traditional analytical assays, and goes on to suggest that microchips that can perform a variety of tests, or "unit operations", on a single sample of cells will vastly increase the knowledge acquired per assay*. He further discusses the importance of being able to scale analytical technologies to handle ever increasing sample sizes. This is a critical barrier to overcome because many concepts are limited in their ability to scale, which impedes the progress of the use of microchips and microfluidic devices within PAT systems. Furthermore, Love gives an example of a scalable, multi-assay chip used to a robust analytical profile of single cells.[34] Love's perspective sets the stage for our investigation of micro and nanoscale devices. As this field of development grows, it will become increasingly necessary for sound engineering principles to be applied so that discrete analytical tests focused on single biological attributes can be effectively combined within a multi-assay device that ensures optimal knowledge acquired per sample.

One growing field of research that is a prime candidate for multifunctional microchips is glycobiology. In particular, increasing the speed and throughput of gly-

*In Chapter 3, acquisition of knowledge is highlighted as one key component for evaluating PAT opportunities.

can assays has gained attention due to the fact that the glycosylation—or lack of glycosylation—of therapeutic proteins has a significant impact on their therapeutic function. Reuel and his coauthors review the burgeoning field of rapid, nanoscale, glycan profiling devices. One of their primary findings is that most of these devices require no labeling or additional steps to detach the glycan from its host protein. Traditional glycan analysis methods such as mass spectrometry, chromatography, and capillary electrophoresis often require such a cleaving step before performing the actual glycosylation assay. Microchips, on the other hand, can provide rapid generation of glycosylation profiles of a protein sample with minimal preparative work. The device reviewed employ various modes of detection such as microscale cantilevers, quartz crystals, and fluorescing carbon nanotubes as methods of determining the glycosylation-related attributes of therapeutic proteins.[35]

Clearly, rapid, high-throughput analytical methods such as these glycosylation profilers are promising candidates for inclusion in an integrated, analytical microdevice that could be used as a future PAT tool for biopharmaceutical manufacturing. One could envision such devices interfacing with bioreactors during protein production to perform rapid assays of cells and proteins which could then determine whether process parameters are appropriate to produce the desired products.

The microscale technologies discussed so far have often been developed and optimized for analyzing the attributes of various aspects of a proteins, cells, or their components. Many of these devices can also be adapted to use the same technological principles to analyze and detect impurities in the biopharmaceutical manufacturing process such as viruses, nucleic acids, and bacteria. In many ways, ensuring that a manufactured therapeutic solution is free of potentially damaging impurities is at least as important as ensuring that the protein and other desired product components meet specifications. Controlling impurities is a critical factor in biopharmaceutical manufacturing, but the topic has previously received little public attention because contaminations can be a sensitive topic for companies to disclose. However, a significant viral contamination at a Genzyme facility and the subsequent product rationing that ensued highlighted the need to understand how impurities contribute to prod-

uct risk.[36] The technology used in the microscale devices described here could also be used in the detection of impurities. In this way, an integrated microscale device or devices could assist in the type of holistic monitoring of the biopharmaceutical manufacturing process described in Chapter 2.

4.3 Technology from other industries and disciplines

We have previously mentioned that biopharmaceutical manufacturing is similar in many ways to other process industries, including oil refining and fermenting yeast. The list of similar industries could also include water purification and treatment, traditional pharmaceutical manufacturing, food processing, and chemical manufacturing. Because individual industries emphasize development of technology at different rates and in different areas, it remains imperative that biopharmaceutical manufacturers and other participants in the biopharmaceutical value chain monitor and leverage developments in other industries and disciplines.

Junker and Wang point out that many of the key developments that led to the current biopharmaceutical PAT initiative came before widespread biopharmaceutical manufacturing based on recombinant DNA-enabled cell lines. Other industries that developed and enhanced the early versions of computer-controlled fermentation were involved in the production of food proteins, enzymes, antibiotics, and organic acids, among others.[20] When new industries, such as biotechnology, are created, they often rely on previously developed technology to enable their operations. It is vital that these industries avoid the trap of becoming too reliant on internally developed technology at the expense of leveraged opportunities for improvement.

For example, our novel PAT system was the result of the sharing of information across industries. The capabilities to modify the high-sensitivity turbidimeter used in the novel PAT system described in Chapter 3 would not have been available had our supplier not developed similar process analyzers for yeast fermentation. Although

these process analyzers were not optimized for our application, the combination of biopharmaceutical, process analysis, and yeast fermentation expertise enabled our team to optimize critical device components such as materials, process connectivity, control system connectivity, radiation source, detection methods, and data transmission. After sharing experience and expertise, we were able to engineer the PAT system so that it would be an ideal solution for our targeted application.

In a similar way, biopharmaceutical manufacturing can benefit from—and contribute to—interactions and knowledge sharing with other industries and disciplines. Other opportunities to combine knowledge regarding process analytical technology for disparate sources will continue to appear in the future. However, in order to take advantage of these opportunities, the biopharmaceutical industry needs to ensure that it employs people with a diverse set of skills and expertise. Knowing what types of skills and abilities are ideal for the development and implementation of new technology can be difficult, and our recommendations can provide some guidance on how best to prepare an operational organization to incorporate PAT systems effectively.

Chapter 5

Recommendations

We have detailed a framework to aid in identifying, evaluating, and implementing PAT opportunities. In addition, we have described a novel PAT system that can be used to analyze and control large-scale chromatography. The novel PAT system clearly demonstrated that opportunities exist for valuable implementation of future PAT systems, for which we will provide our recommendations. In light of these results, these recommendations align with five major categories: a simple PAT strategy message, opportunity identification, system evaluation, chromatography analysis and control, and areas for future research.

As discussed in Chapter 2, a critical factor to the success of any organizational PAT deployment is starting with a simple message. For this reason, we recommend that organizations communicate that *PAT manages product risk throughout the pharmaceutical manufacturing value chain through the innovative, effective acquisition and application of data*. The importance of clearly communicating new ideas or projects throughout an organization is sometimes overlooked, and for this reason we encourage PAT adopters to ensure that they invest the time and effort necessary to effectively communicate the rationale behind PAT.

For opportunity identification, we recommend that a holistic, two-dimensional approach such as that described in Chapter 3 be adopted. It is not sufficient to simply examine discrete unit operations within a biopharmaceutical manufacturing facility in an effort to identify areas for PAT improvement. The focus should be

on actively synthesizing information from both laboratory assays and manufacturing unit operations in order to find ways to improve the overall capability of integrated facilities to manage product risk. While these opportunities may arise from individual operations, many beneficial opportunities will result from combining operations into continuous flow or augmenting process control by incorporating offline assays into a PAT system.

We further recommend that potential PAT systems be evaluated according to the criteria of quality, cycle time, yield, process knowledge, and cost. By employing these criteria, PAT systems can be effectively implemented in biopharmaceutical operations and enhance the risk management capabilities of the facility. Process knowledge is not always a commonly used criterion, but as we discussed in Chapter 4 the increased yield of process knowledge per sample analyzed can greatly benefit biopharmaceutical manufacturing.

We recommend the novel PAT system presented in Chapter 3 for analyzing and controlling large-scale chromatography, specifically chromatography that targets the reduction of high molecular weight species (HMWS). Using a light scattering analyzer and UV spectrophotometer enables online analysis of average molecular weight in the process stream, which correlates to the cumulative % HMWS processed (under certain conditions). While this may not be the ideal solution for every chromatography step, it is especially advantageous given its simplicity, speed, and direct measurement of average molecular weight as compared to alternative chromatography control methods.

Finally, we recommend that further process analytical technology research focus on advanced optical technologies, microscale and nanoscale devices, and sharing knowledge with other industries and disciplines. This tripartite approach will increase the efficacy of research efforts and develop technologies that are optimized for use as PAT systems. Through a focused effort on developing the appropriate systems and organizational capabilities necessary to optimize the use of PAT, biopharmaceutical manufacturers will be poised to overcome the challenges currently facing the industry.

Chapter 6

Industry Implications and Conclusion

Numerous market pressures are urging the biopharmaceutical industry to abandon operating regimes associated with the historical blockbuster-oriented business model, such as a focus on building capacity. In turn, these pressures are encouraging a greater emphasis on on operational excellence. Biopharmaceutical manufacturers and regulatory agencies are assessing how these pressures will change the landscape of biopharmaceutical manufacturing, and they have concluded that Process Analytical Technology (PAT) is a crucial component of a plan to increase yield, quality, and knowledge in coming years.

The implementation of PAT will have significant implications for the biopharmaceutical industry, particularly in terms of operations. The first major implication will be assessing and filling gaps in the skill level of personnel. Effective process analysis will not significantly increase the amount of data acquired for the sake of having more data, but will significantly increase the amount of process data relevant to managing risk. Even in the ideal case, the increase in relevant data will require the industry to train and hire people who are capable of analyzing, processing, and making sound judgments based on this data. This change in skills required to effectively manufacture biopharmaceuticals will require the industry to adapt its workforce accordingly. Consequently, not only will industry need to enhance its ability to train and obtain

these abilities, but educational institutions will need to continually improve curricula to supply the market with skilled personnel.

Another important industry implication resulting from the increase in the use of PAT involves the systems that support, maintain, and transmit the process analysis information. For example, biological processes are known for having a higher number of measurable input parameters, process variables, and output characteristics than many other process industries. However, the measurements of process variables and output characteristics can be slow and difficult to perform reliably. In contrast, many of the information systems and automation platforms currently available to the biopharmaceutical industry have been designed using a philosophy better suited to other industries. Therefore, to ensure that PAT delivers value, the biopharmaceutical industry needs to ensure that information systems and automation platforms are designed to accommodate its manufacturing process. These systems will need to be capable of handling the high number of variables and unique forms of measurement characteristic of biopharmaceutical manufacturing.

Because these changes are still occurring in the biopharmaceutical industry, it is generally accepted that current methods for measuring and quantify biological processes are limited and the lack of sufficiently robust methods is slowing discovery of beneficial therapeutics. We believe that by emphasizing and investing in Process Analytical Technology, both biopharmaceutical manufacturing and bioanalytical sciences can benefit from the ensuing developments. It sometimes seems counterintuitive that large-scale commercial manufacturing can be a source of innovation and expanded knowledge because it can be perceived as cumbersome and rote. However, certain aspects of manufacturing, such as high volumes and the drive for consistent quality, have historically enabled analytical tools to provide and confirm valuable insights into the critical attributes of processes and products that otherwise would have gone unnoticed.

Process Analytical Technology, in many ways, is still in the early stages of implementation in the biopharmaceutical industry even a decade after concerted initiatives by regulatory agencies. As we have noted in previous chapters, there are challenges

and limitations associated with the implementation of PAT systems, and such challenges also accompany research into new possibilities. However, in spite of these difficulties, the quantifiable benefits of effectively deploying analytical technology in industrial processing are well documented. By appropriately identifying, evaluating, and implementing PAT systems, the biopharmaceutical industry can ensure that it continues to supply valuable therapies to the patients who need them.

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Appendix A

Supplemental Information

This appendix contains supplemental information for the experiments conducted during this research. The tabulated data for each run shows only the relevant data from the beginning of detectable protein elution until the accumulated material contains approximately 3–4% HMWS. Run 1 had a flow rate of 0.8 L/min, while the other runs were maintained at a flow rate of 2 L/min. Run 2 is not included in Figure 3-10 because it is an outlier.

Also, for Run 4 we did not back-calculate LS prior to peak max; we had fully automated the calculation routine by that run so that $\overline{M}$ was only calculated by the DCS system once peak max was reached. The % HMWS in pool measurements have a method RSD of 3.87%, but additional error could arise from mixing collected sample vials as described in Section 3.4.3. Similarly to Section 3.4.4, in this appendix $UV = \frac{UV}{UV,maximum}$, $I_S = \frac{I_S}{I_S,maximum}$, and $\overline{M} = \frac{\overline{M}}{\overline{M},monomer}$, making them unitless quantities.

Time (hr)	UV	I_S	$\overline{M}$	% HMWS in pool
0.00	0.090	0.090	1.000	0.100
0.05	0.593	0.594	1.002	0.186
0.10	0.850	0.849	0.998	0.137
0.15	0.963	0.963	1.000	0.163
0.20	0.996	0.996	1.000	0.174
0.25	1.000	1.000	1.000	0.180
0.30	0.979	0.979	1.000	0.203
0.35	0.940	0.940	1.000	0.249
0.40	0.880	0.878	0.999	0.306
0.45	0.801	0.798	0.997	0.358
0.50	0.716	0.712	0.995	0.413
0.55	0.622	0.622	1.001	0.523
0.60	0.502	0.518	1.030	0.750
0.65	0.369	0.419	1.136	1.206
0.70	0.273	0.361	1.323	1.977
0.75	0.210	0.314	1.492	2.875
0.80	0.158	0.257	1.621	3.715
0.85	0.118	0.206	1.747	4.322

Table A.1: Tabulated experimental data from run 1

Time (hr)	UV	I_S	$\overline{M}$	% HMWS in pool
0.00	0.231	0.235	1.017	0.000
0.05	0.533	0.554	1.039	0.080
0.10	0.736	0.749	1.017	0.091
0.15	0.875	0.882	1.008	0.095
0.20	0.926	0.925	0.998	0.097
0.25	0.964	0.960	0.996	0.097
0.30	0.979	0.973	0.994	0.098
0.35	1.000	1.003	1.003	0.098
0.40	0.991	0.995	1.004	0.098
0.45	0.980	0.987	1.007	0.099
0.50	0.977	0.983	1.006	0.099
0.55	0.978	0.988	1.010	0.109
0.60	0.961	0.969	1.008	0.108
0.65	0.951	0.967	1.016	0.116
0.70	0.925	0.938	1.015	0.122
0.75	0.861	0.888	1.032	0.128
0.80	0.781	0.810	1.038	0.132
0.85	0.675	0.707	1.047	0.141
0.90	0.544	0.578	1.062	0.152
0.95	0.414	0.435	1.050	0.166
1.00	0.327	0.348	1.064	0.192
1.05	0.267	0.297	1.114	0.247
1.10	0.234	0.294	1.258	0.374
1.15	0.221	0.316	1.427	0.654
1.20	0.207	0.333	1.603	1.122
1.25	0.171	0.305	1.778	1.751
1.30	0.138	0.249	1.808	2.390
1.35	0.118	0.233	1.972	2.917
1.40	0.101	0.215	2.131	3.371

Table A.2: Tabulated experimental data from run 2

Time (hr)	UV	I_S	$\overline{M}$	% HMWS in pool
0.00	0.079	0.091	1.150	0.200
0.05	0.393	0.406	1.034	0.283
0.10	0.709	0.713	1.006	0.356
0.15	0.897	0.897	1.000	0.376
0.20	0.967	0.961	0.994	0.384
0.25	0.983	0.982	0.998	0.388
0.30	0.992	0.987	0.995	0.390
0.35	0.999	0.999	0.999	0.392
0.40	0.972	0.973	1.001	0.393
0.45	0.962	0.968	1.007	0.394
0.50	0.967	0.968	1.001	0.395
0.55	0.948	0.953	1.006	0.405
0.60	0.940	0.946	1.006	0.413
0.65	0.927	0.933	1.006	0.420
0.70	0.893	0.898	1.007	0.433
0.75	0.845	0.843	0.998	0.450
0.80	0.791	0.788	0.996	0.469
0.85	0.724	0.720	0.994	0.490
0.90	0.666	0.663	0.995	0.521
0.95	0.599	0.598	0.998	0.564
1.00	0.549	0.550	1.002	0.633
1.05	0.482	0.486	1.009	0.765
1.10	0.403	0.425	1.055	0.988
1.15	0.322	0.376	1.169	1.347
1.20	0.269	0.353	1.310	1.837
1.25	0.233	0.335	1.438	2.403
1.30	0.195	0.299	1.528	2.871
1.35	0.164	0.270	1.645	3.285
1.40	0.143	0.252	1.762	3.625

Table A.3: Tabulated experimental data from run 3

Time (hr)	UV	I_S	$\overline{M}$	% HMWS in pool
0.00	0.059			0.100
0.05	0.388			0.192
0.10	0.683			0.197
0.15	0.862			0.198
0.20	0.910			0.199
0.25	0.930			0.199
0.30	0.943			0.199
0.35	0.965			0.199
0.40	0.969			0.200
0.45	0.976			0.200
0.50	0.995	0.998	1.003	0.200
0.55	0.984	0.992	1.009	0.200
0.60	0.956	0.964	1.009	0.209
0.65	0.925	0.935	1.010	0.217
0.70	0.877	0.877	1.000	0.223
0.75	0.792	0.791	0.998	0.234
0.80	0.710	0.705	0.993	0.242
0.85	0.632	0.625	0.988	0.254
0.90	0.559	0.551	0.984	0.271
0.95	0.494	0.487	0.986	0.317
1.00	0.444	0.439	0.989	0.392
1.05	0.388	0.392	1.010	0.530
1.10	0.328	0.349	1.064	0.784
1.15	0.282	0.332	1.176	1.211
1.20	0.247	0.326	1.320	1.772
1.25	0.218	0.315	1.443	2.388
1.30	0.187	0.285	1.527	2.927
1.35	0.158	0.257	1.622	3.395
1.40	0.130	0.224	1.722	3.786

Table A.4: Tabulated experimental data from run 4

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Appendix B

Recommended Reading

This appendix contains recommended reading for those looking for introductory materials about the various aspects of Process Analytical Technology in biopharmaceutical manufacturing.

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